COPY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	Group Art Unit: 1631
Michael Grunstein et al.	Examiner: J. Lundgren
Serial No. 09/261,104	Response to Paper No. 9
Filed: March 3, 1999	
For: COMPOSITIONS AND METHODS FOR THE TREATMENT OF ASTHMA	

DECLARATION OF MICHAEL GRUNSTEIN AND HAKON HAKONARSON

We, Michael Grunstein and Hakon Hakonarson, hereby declare that:

1. We are the inventors of the invention described and claimed in U.S. Patent Application Serial No. 09/261,104 (the '104 application).

2. We have read and are familiar with the contents of the Official Action dated August 30, 2000 in the '104 application. We note that the Examiner has rejected claims 1-4 under 35 U.S.C. §102(e) as allegedly anticipated by US Patent 6,011,138 to Reff et al. (hereinafter the '138 application). The purpose of this declaration is to establish that the invention described and claimed in the '104 application (the "subject invention") was conceived of prior to the February 20, 1997, which upon information and belief, is the earliest effective filing date of the '138 application and diligently and constructively reduced to practice at least as early as March 3, 1998, the filing date of the provisional application from which the instant application claims priority under 35 U.S.C. §119(e).

3. Conception of the invention described in the present application is evidenced by a copy of an abstract (Exhibit A)

and copies of notebook pages submitted herewith as Exhibit B1-B7. The dates on the data have been masked for the purpose of this Declaration. Described in the notebook pages are experiments utilized in the development of methods for the treatment of asthma. The present inventors observed that airway smooth muscle tissue (ASM) treated with atopic asthmatic serum demonstrates increased constrictor and reduced relaxation response to acetylcholine and isoproterenol when compared to control serum-treated tissue. These results are described in Exhibit A which is an abstract submitted to ALA/ATS International Conference prior to the earliest effective filing date of the '138 patent. The data in Exhibits B1-B7 reveal that the effects of atopic asthmatic serum are blocked or prevented by preincubation of ASM with an anti-CD23 monoclonal antibody. B1 is a graph of the tracing data presented in Exhibits B6 and B7. The experiments presented in Exhibits B1-B7 were also performed prior to February 20, 1997. Conception of the subject matter described in Exhibits A and B occurred in the United States.

4. Attached hereto as Exhibits C1 and C2 are pages from our laboratory notebooks describing Southern blots showing that the FcεII receptor (CD23) is expressed in airway smooth muscle cells and that this expression is upregulated by exposing the cells to atopic asthmatic serum. These results were observed in both human (C1) and rabbit (C2) tissue. We performed these experiments and contemporaneously recorded the data set forth in the laboratory notebook pages of Exhibit C. The dates appearing in Exhibit C have been masked for the purpose of this Declaration.

5. The results of flow cytometry analysis of CD23 expression are shown in Exhibits D1-D3. Airway smooth muscle tissue was exposed to atopic asthmatic serum for 24 hours. The data show that relative to isotype negative control

antibody (D1) CD23 expression was dramatically upregulated in human asthmatic serum-treated human airway cells (D2) relative to control serum-treated cells (D3).

6. Exhibit E shows the results of experiments performed using airway smooth muscle tissue obtained from human patients. Representative Southern blots are shown revealing that, in contrast to lung tissue, a dramatic increase in CD23 expression is seen in airway smooth muscle tissue of asthmatic patients relative to control non-asthmatic patient tissue.

7. In order to show a reduction to practice of our invention, Exhibit F is attached hereto. Exhibit F is a copy of the provisional application filed March 10, 1998 from which the instant application claims priority under 35 U.S.C. §119(e).

8. After conceiving the invention and carrying out the experimentation described in paragraphs 3-6 above, we were diligent in reducing the invention to practice, including the period from just prior to February 20, 1997 until the invention was subsequently reduced to practice as set forth in paragraph 7 above. The Exhibits described in paragraphs 3-6 describe our work during the relevant period. Thus, we conceived of the present invention prior to the filing date of the '138 application and thereafter diligently reduced the claimed invention to practice.

9. All of the experiments, the results of which are presented in the Exhibits attached to this Declaration, were performed by us or under our direction and supervision.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed true; and further

COPY

that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the above-referenced application or any patent issued thereon.

April 19, 2001
DATE

Michael M. Grunstein, M.D., Ph.D.

April 19, 2001
DATE

Hakon Hakonarson, Ph.D.

1997 ABSTRACT FORM

ALL ABSTRACTS MUST BE IN THE ATS OFFICE BY FRIDAY, NOVEMBER 1, 19
• Papers received after that date will be returned to the author.

FIRST AUTHOR (Please type information)

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ALTERED Fc RECEPTOR EXPRESSION IN ATOPIC/ASTHMATIC SENSITIZED RABBIT AIRWAY SMOOTH MUSCLE. H. Hakonarson, D.J. Herrick, and M.M. Grunstein. Division of Pulmonary Medicine, Joseph Stokes, Jr. Research Institute, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We have previously shown that passive sensitization of rabbit airway smooth muscle (ASM) with human atopic/asthmatic serum produces heightened ASM constrictor responsiveness to acetylcholine (ACh) and attenuated ASM relaxant responsiveness to ß-adrenoceptor stimulation. These modulatory effects of atopic/asthmatic serum on the ASM responsiveness were blocked by depleting the serum of immune complexes and were simulated by exogenous administration of immunoglobulin (Ig) G and IgE immune complexes to naive ASM, suggesting that these functional alterations in responsiveness in the sensitized state were mediated by activation of Fc receptors on the ASM. The present study extended these earlier observations to examine whether rabbit ASM cells express Fc receptors and whether the expression of Fc receptors on ASM is modulated by atopic asthmatic serum. Fc receptor expression was determined by: 1) RT-PCR and southern blotting using human-specific Fcγ RI, RII and RIII and Fcε RII primers, and cDNA probes; and 2) flow cytometry using human monoclonal antibodies specific for the individual Fc receptors. cDNA was synthesized from cultured ASM cells following 0, 3, 6 and 24 hr treatment with atopic/asthmatic vs. control serum. Relative to the immortalized 8.1.6 B-cell line, ASM cells expressed mRNA for the Fcγ RIII and the Fcε RII receptors at relatively low levels and their mRNA expression did not change significantly during treatment of the cells with atopic/asthmatic serum. Similarly, ASM cells stained positive for cell surface Fcγ RI, -RII, -RIII receptor expression (40-80%), and their expression of these receptors did not change significantly during treatment with the atopic asthmatic serum. In contrast, expression of the Fcε RII receptor, although present at a low level (15%) in control ASM, was enhanced approximately two fold following treatment with the atopic/asthmatic serum. Collectively, these observations provide new evidence demonstrating that rabbit ASM cells express Fcγ and Fcε RII receptors, and that the cell surface expression of the low-affinity receptor for IgE (Fcε RII) is significantly enhanced by exposure to atopic/asthmatic serum. These findings support the concept that Fc receptors on ASM may mediate the effects of atopic sensitization on ASM responsiveness.

This abstract is funded by: NIH Grant HL-31467; Parker B. Francis Fellowship Award.

Presentation Format (please check one): ☑ either slide or poster ☐ poster only

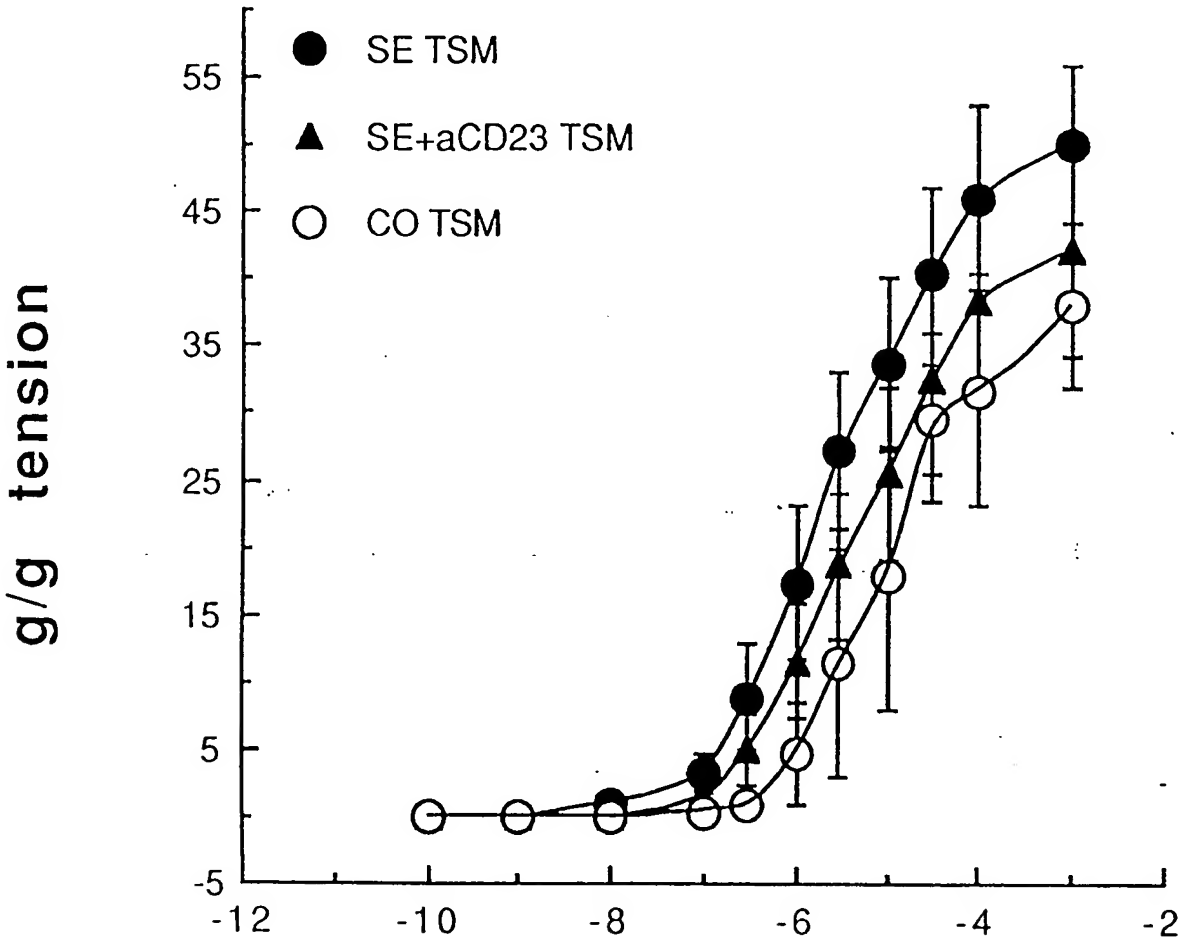
- Processing fee is $35.00. Indicate method of payment on attached form.
- Each abstract must be submitted on an original form.
- Three 8 1/2" x 11" copies of the abstract page must also be submitted.
- Abstract print must be black.
- Type must be no smaller than 10 point.
- Abstract must not go beyond the blue box.
- Set margins at: Top = 3" (7.5 cm); Left = 1" (2.5 cm)
- The submission of multiple abstracts from the same laboratory with closely related data is discouraged.
- An abstract should not be submitted if it is to be published or presented at a major national or international meeting prior to the 1997 International Conference.

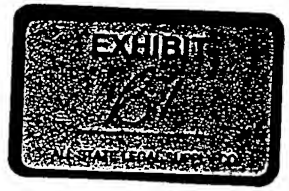
EXHIBIT A

CATEGORY # 35 (Indicate category number that best fits your abstract.)

▶ To remove page, fold and tear along dotted line ◀

Acetylcholine DRC Se/Se+aCD23/CO TSM





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1	Date:											Calibration	0 2				
2	Expt:								ACH/KCL paper-template PG								
3																	
4		BL/Tis Wt		[-9]	[-8]	[-7]	[3x]	[-6]	[3x]	[-5]	[3x]	[-4]	[-3]	Tmax KCl			
5		Reading	6 00	6 00	6 30	6 50	7 50	10 30	16 50	20 50	25 00	29 00	33 00				
6	TSM#1	Ten-Base		0 00	0 30	0 50	1 50	4 30	10 50	14 50	19 00	23 00	27 00				
7		g/g Tis	0 132	0 00	0 45	0 76	2 27	6 52	15 91	21 97	28 79	34 85	40 91	~~26 78~~	[3X10-7]		
8	SE			0 00	1 11	1 85	5 56	15 93	38 89	53 70	70 37	85 19	100 00				
9		ACh/KCl		0 00	1 70	2 83	8 48	24 32	59 38	82 01	107 46	130 08	152 70				
10																	
11		Reading	6 00	6 00	6 50	8 30	12 50	16 50	20 50	24 00	27 30	30 00	31 00				
12	TSM#2	Ten-Base		0 00	0 50	2 30	6 50	10 50	14 50	18 00	21 30	24 00	25 00				
13	SE	g/g Tis	0 082	0 00	1 22	5 61	15 85	25 61	35 37	43 90	51 95	58 54	60 98	~~6 83~~	[3X10-7]		
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15		ACh/KCl		0 00	6 66	82 13	232 12	374 96	517 80	642 79	760 63	857 05	892 76				
16																	
17		Reading	9 00	9 00	9 50	10 30	12 50	17 50	22 00	24 00	26 00	28 00	29 50				
18	TSM#3	Ten-Base		0 00	0 50	1 30	3 50	8 50	13 00	15 00	17 00	19 00	20 50				
19	SE	g/g	0 085	0 00	1 18	3 06	8 24	20 00	30 59	35 29	40 00	44 71	48 24	~~7 73~~	[3X10-7]		
20		%max		0 00	2 44	6 34	17 07	41 46	63 41	73 17	82 93	92 68	100 00				
21		ACh/KCl		0 00	15 22	39 57	106 54	258 73	395 71	456 59	517 46	578 34	624 00				
22																	
23		Reading	8 00	8 00	8 00	8 00	8 00	10 50	14 50	18 50	23 50	28 00	31 00				
24	TSM#4	Ten-Base		0 00	0 00	0 00	0 00	2 50	6 50	10 50	15 50	20 00	23 00				
25	SE +	g/g	0 152	0 00	0 00	0 00	0 00	3 29	8 55	13 82	20 39	26 32	30 26	0 86	[10-7]		
26	αCD_{63}	%max		0 00	0 00	0 00	0 00	10 87	28 26	45 65	67 39	86 96	100 00				
27		ACh/KCl		0 00	0 00	0 00	0 00	382 50	994 49	1606 49	2371 48	3059 98	3518 97				
28																	
29		Reading	8 50	8 50	8 50	9 50	13 50	18 50	23 50	28 50	33 00	37 50	39 50				
30	TSM#5	Ten-Base		0 00	0 00	1 00	5 00	10 00	15 00	20 00	24 50	29 00	31 00				
31	SE +	g/g	0 110	0 00	0 00	1 82	9 09	18 18	27 27	36 36	44 55	52 73	56 36	10 42	[10-7]		
32	αCD_{63}	%max		0 00	0 00	3 23	16 13	32 26	48 39	64 52	79 03	93 55	100 00				
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34																	
35		Reading	6 00	6 00	6 30	7 00	9 00	12 50	16 00	19 00	22 00	24 00	25 50				
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39		ACh/KCl		0 00	4 67	15 56	46 69	101 17	155 64	202 33	249 02	280 15	303 50				
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43		g/g	0 100	0 00	0 00	0 00	0 00	1 00	3 00	8 00	16 00	23 00	32 00	40 68	CONTROL		
44	CO	%max		0 00	0 00	0 00	0 00	3 13	9 38	25 00	50 00	71 88	100 00				
45		ACh/KCl		0 00	0 00	0 00	0 00	2 46	7 37	19 67	39 33	56 54	78 66				
46																	
47		Reading	9 00	9 00	9 00	9 50	10 00	14 50	22 00	27 00	32 50	35 50	38 00				
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49		g/g	0 131	0 00	0 00	0 76	1 53	8 40	19 85	27 48	35 88	40 46	44 27	~~39 7~~	CONTROL		
50	Co	%max		0 00	0 00	1 72	3 45	18 97	44 83	62 07	81 03	91 38	100 00				
51		ACh/KCl		0 00	0 00	1 92	9 85	21 15	49 99	69 22	90 37	101 91	111 52				
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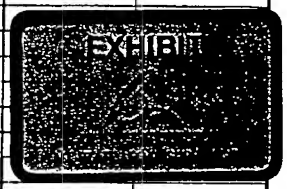
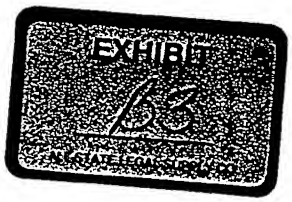


Rabbit 1899 DENFC : ALL DRC

	-10	-9	-8	-7	-6.52	-6	-5.52	-5	-4.52	-4	-3
	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}
1	0	0	.450	.760	2.270	6.520	15.910	21.970	28.790	34.850	40.910
2	0	0	1.220	5.610	15.850	25.610	35.370	43.900	51.950	58.540	60.980
3	0	0	1.178	3.060	8.240	20.000	30.590	35.290	40.000	44.710	48.240
4	•	•	•	•	•	•	•	•	•	•	•
5	0	0	.949	3.143	8.787	17.377	27.290	33.720	40.247	46.033	50.043
6	0	0	.250	1.401	3.930	5.665	5.855	6.379	6.687	6.871	5.863



	-10	-9	-8	-7	-6.52	-6	-5.52	-5	-4.52	-4	-3
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
1	0	0	0	0	0	3.290	8.550	13.820	20.390	26.320	30.260
2	0	0	0	1.820	9.090	18.180	27.270	36.360	44.550	52.730	56.360
3	0	0	0	2.020	6.060	13.130	20.200	26.260	32.320	36.360	39.390
4	•	•	•	•	•	•	•	•	•	•	•
5	0	0	0	1.280	5.050	11.533	18.673	25.480	32.420	38.470	42.003
6	0	0	0	.643	2.672	4.372	5.458	6.518	6.975	7.697	7.647

EXHIBIT

ALL-STATE LEGAL SUPPLY CO.

Rabbit body contour : pitch

	-10	-9	-8	-7	-6.52	-6	-5.52	-5	-4.52	-4	-3
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
1	•	•	•	•	•	•	•	•	•	•	•
2	0	0	0	0	0	1.000	3.000	8.000	23.500	23.000	32.000
3	0	0	0	.760	1.530	8.400	19.850	27.480	35.880	40.460	44.270
4	•	•	•	•	•	•	•	•	•	•	•
5	0	0	0	.380	.765	4.700	11.425	17.740	29.690	31.730	38.135
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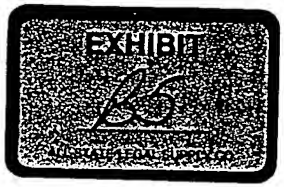

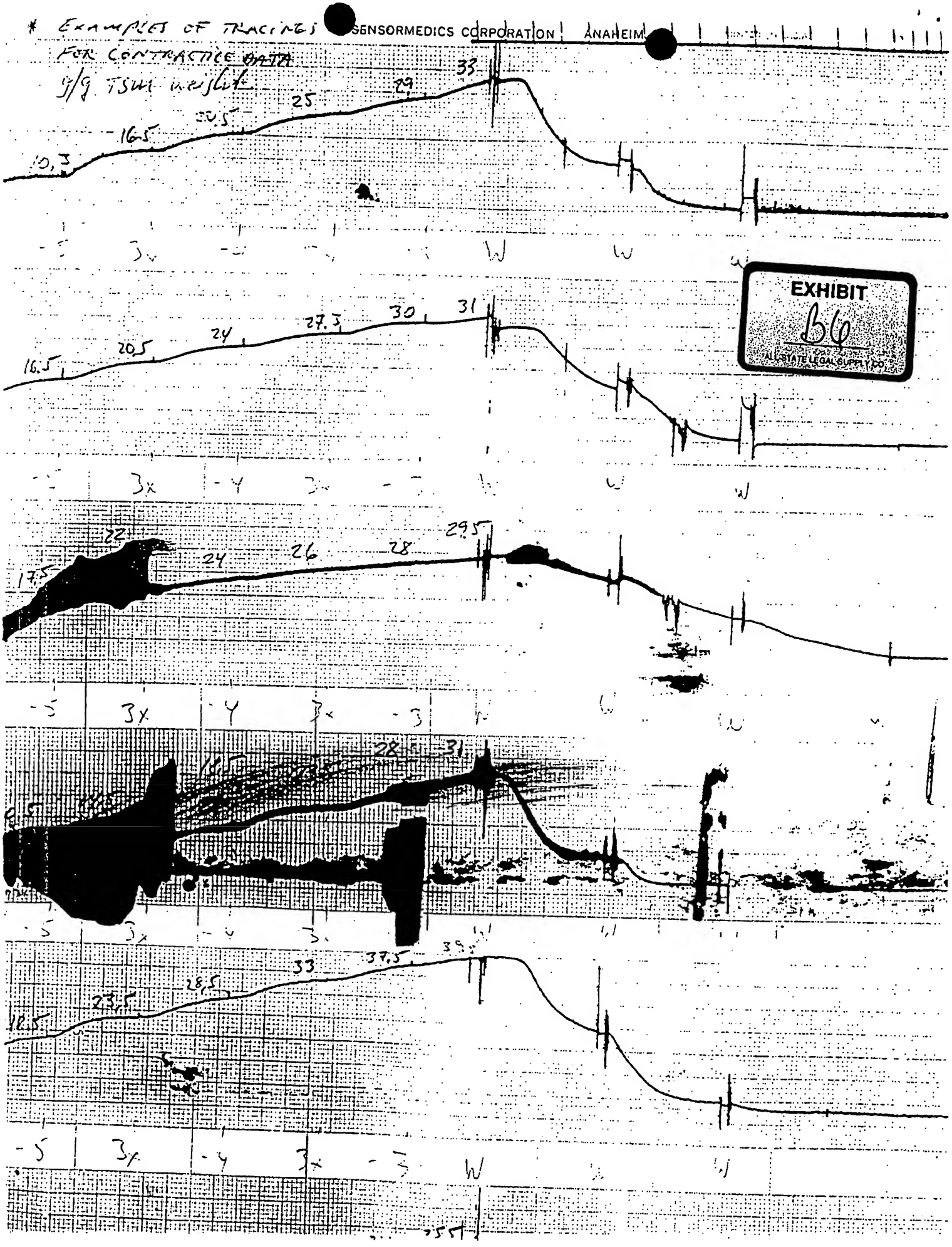
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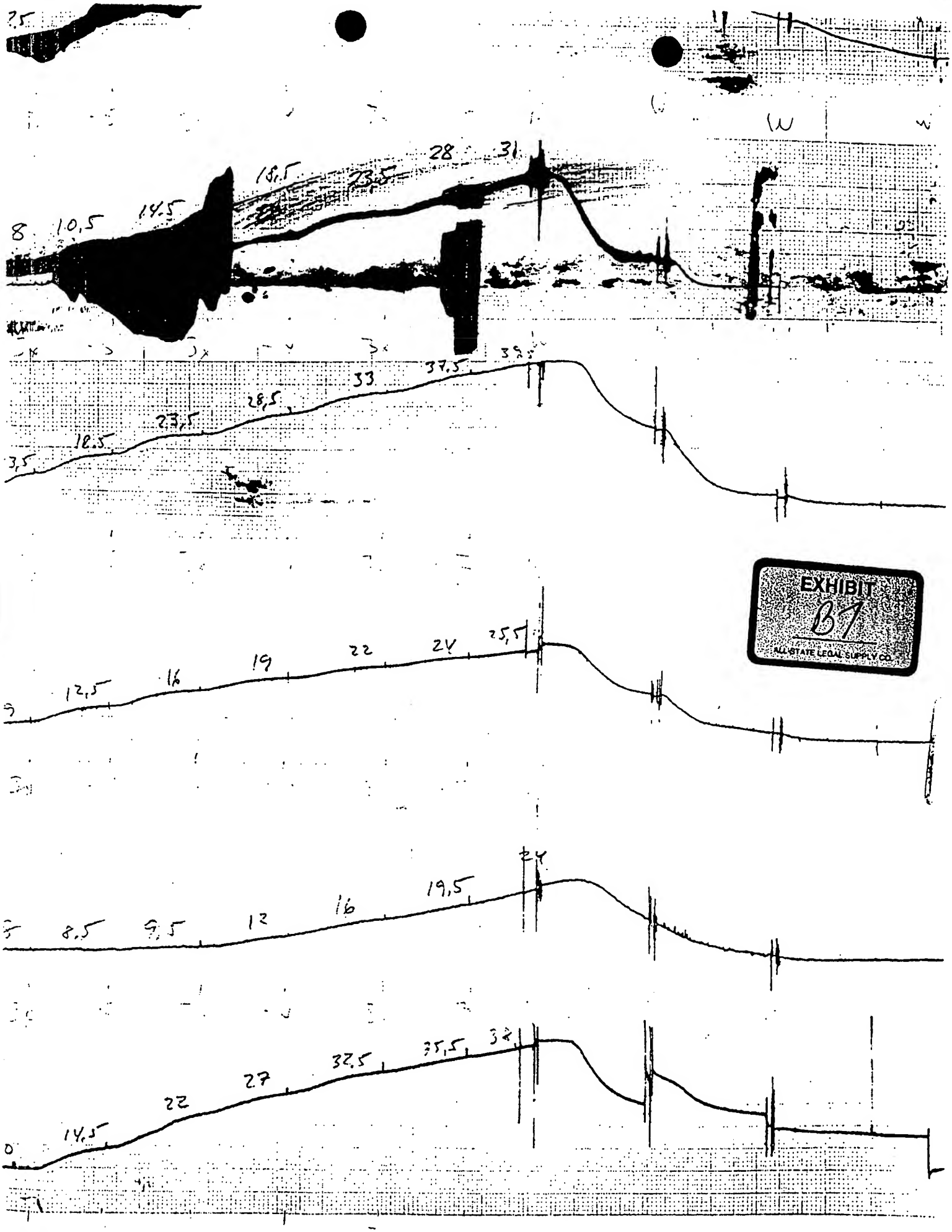
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FOR - ON GEL

(1) HBSM CONTROL 6° = Lane

(2) HTSM SENSIT 6° = Lane

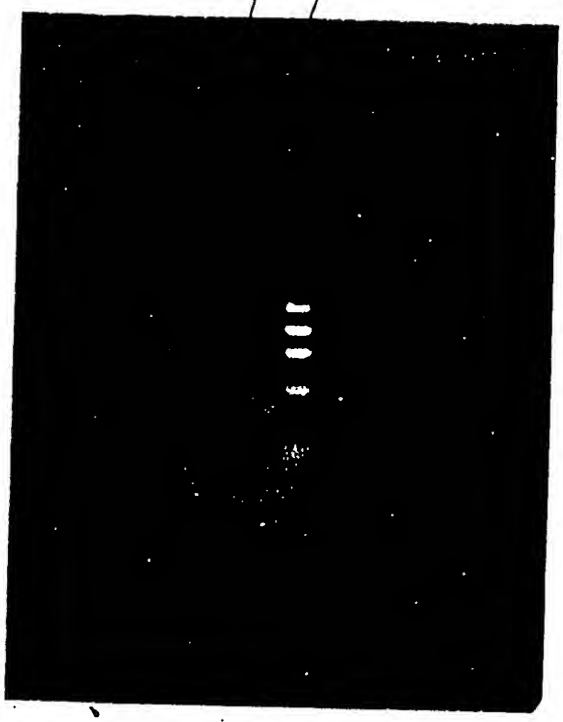
(3) HBSM CONTROL 6° = ——— : 5

(4) HBSM Sensitive 6° = ——— : 6

(5) Φx-174 MARKER = ——— : 7

(1.5 µl marker + 5.2 µl 0.1 X TAE)

+ µl dye.



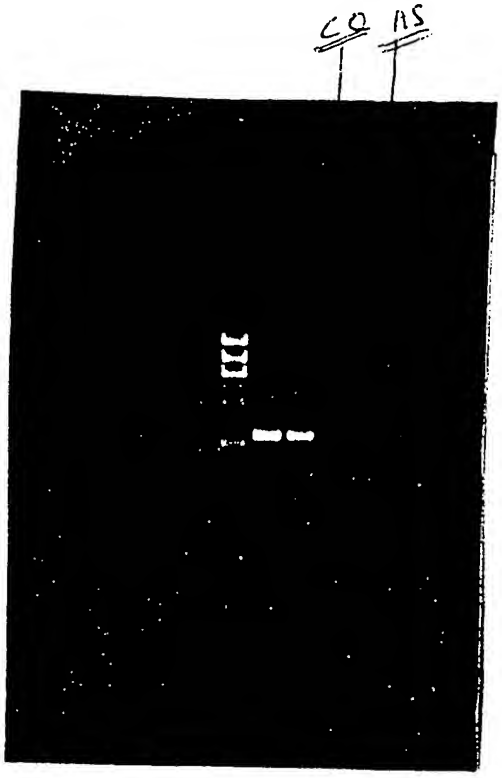
ORDER ON 30ml ; 1.2% AGAROSE
1X TAE MINI-GEL

(1) IGFBP-2 PCR Q FRAG (H_2O)
(2) " " " " (TRIS)
(3) IGFBP-3 " " " (H_2O)
(4) " " " " (TRIS)

} 2.5ul DNA

(5) ΦX 174 c HaeIII 2ul DNA
(6) GHR CONTROL RTSM } IL-1β PRIMERS
(7) " ASTHM. " }
(8) " CONTROL " } FcERI PRIMERS
(9) " ASTHM. " }

} 5ul DNA



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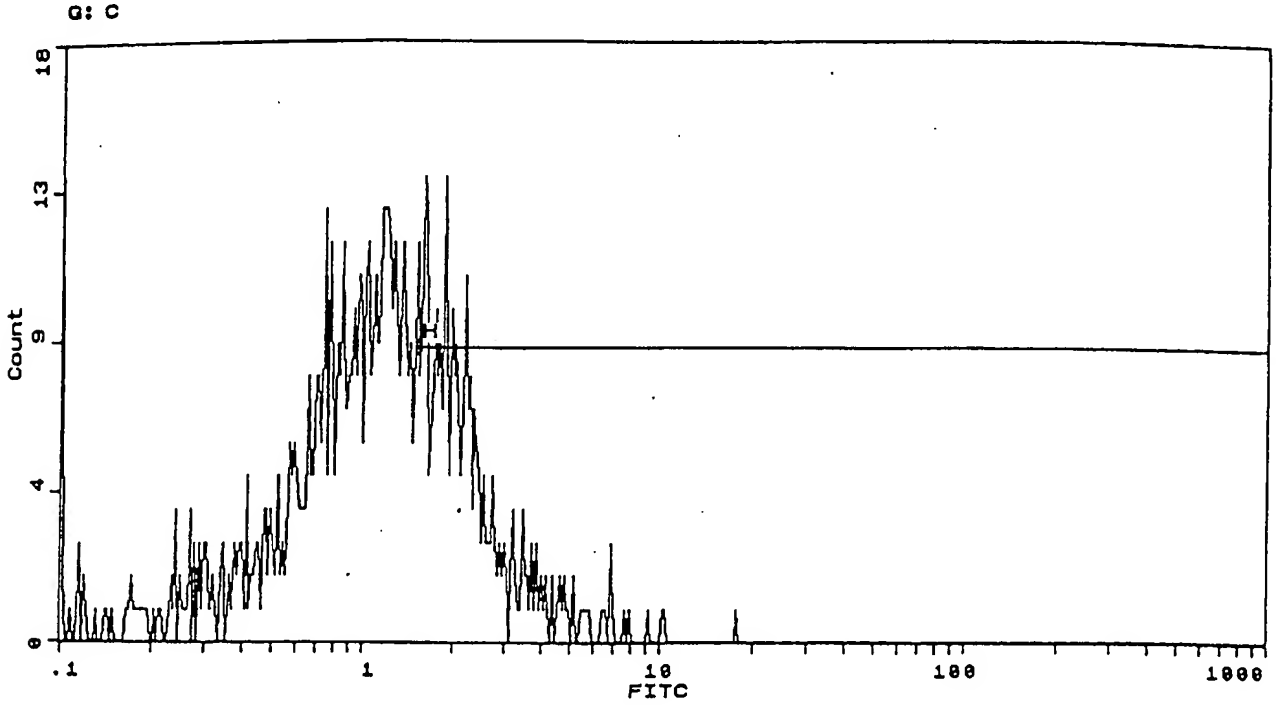
SAMPLE DATE: 07Oct96
SAMPLE TIME: 11:51:59



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SAMPLE NUMBER:	05/03/96			SAMPLE TIME:	11:51:59
COMMENTS	:				

---------- STATISTICS ----------

SINGLE PARAMETER STATISTICS

			......Peak......		.....X Channel.....					
ID	Pcnt	Area	Position	Height	Mean	SD	FullCV	HalfCV	Min	Max
H	36.2	724	1.6	18	2.58	1.46	56.6	2.15	1.5	1024

H Low Channel = 1.5, High Channel = 1024 H

CD23 ; Sonified -

GATES

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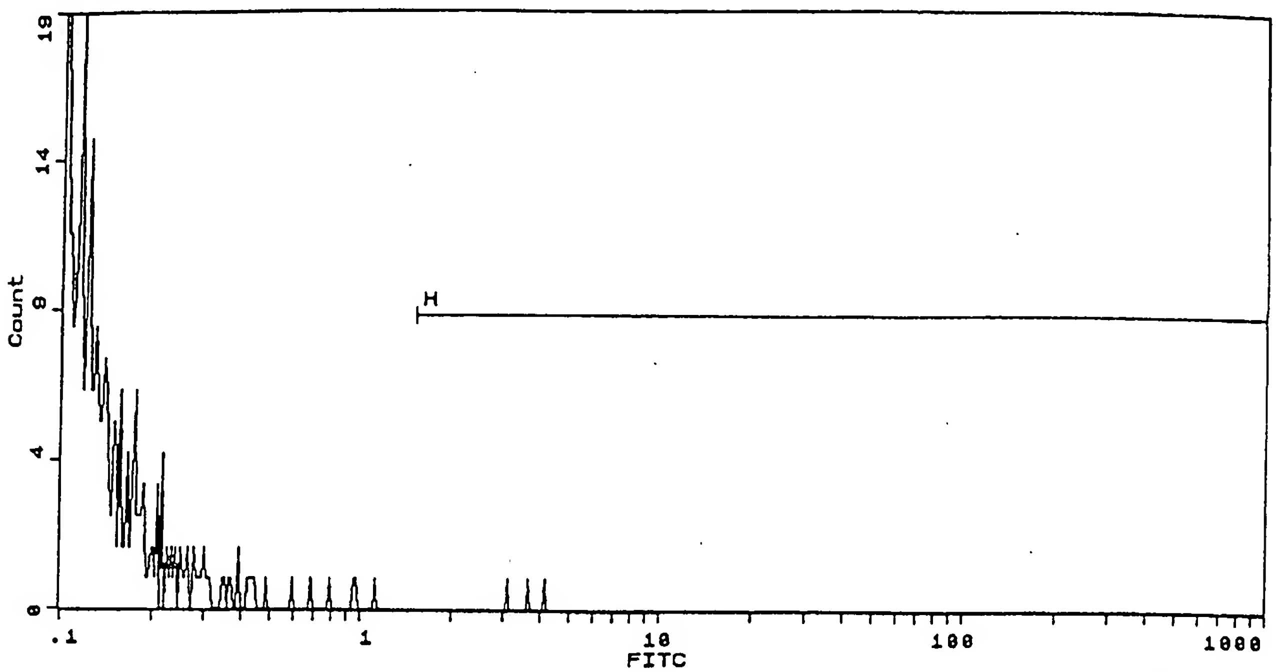
SAMPLE NUMBER:

SAMPLE DATE
SAMPLE TIME: 11:53:57

G: C



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SAMPLE NAME : 1A
SAMPLE NUMBER: 05/03/96
COMMENTS :

HIST COUNT : 2,000

LIST FILE : HAKON12.LMD
PROT FILE : HB000049.PRO
SAMPLE DATE:
SAMPLE TIME:

-- STATISTICS --

SINGLE PARAMETER STATISTICS

ID	Pcnt	Area	......Peak...... Position	Height	..............X Channel.............. Mean	SD	FullCV	HalfCV	Min	Max
H	1.2	23	3.2	2	3.07	1.30	42.2	0.382	1.5	1024

H Low Channel = 1.5, High Channel = 1024 H

Fig 6. 15 mpo control sintered

SAMPLE NAME : IGG1 FITC CT
SAMPLE NUMBER:

GATES

SAMPLE DATE:
SAMPLE TIME: 14:39:28

G: A

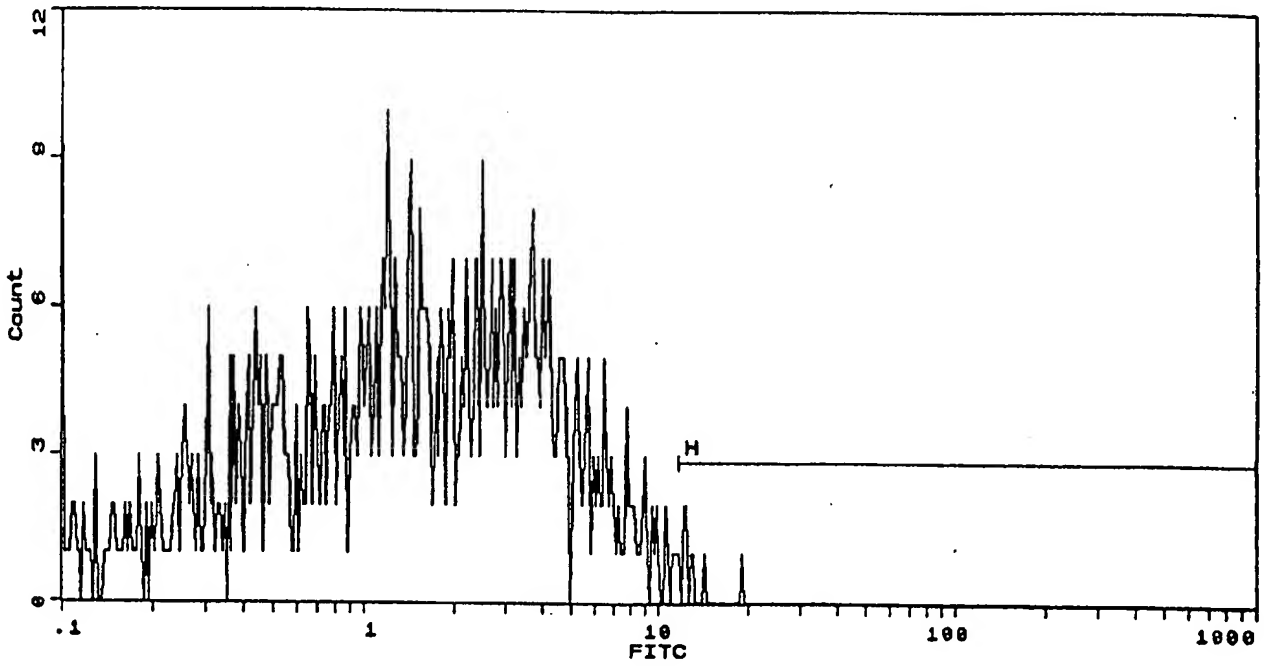

EXHIBIT
D3
ALL-STATE LEGAL SUPPLY CO.

---------- SAMPLE INFO ----------

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SAMPLE NAME	: IGG1 FITC CT			SAMPLE DATE:	
SAMPLE NUMBER:				SAMPLE TIME:	
COMMENTS	:				

---------- STATISTICS ----------

SINGLE PARAMETER STATISTICS

		Peak		X Channel					
ID	Pcnt	Area	Position	Height	Mean	SD	FullCV	HalfCV	Min	Max
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OF: Michael M. Grunstein et al.

FOR: Compositions and Methods for Treatment of Asthma

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INVENTOR(s) APPLICANT(s)

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TITLE OF INVENTION (280 characters maximum)

Compositions And Methods For Treatment of Asthma

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The invention was made by an agency of the United States Government or under a contract with an agency of the U.S. Government.

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Respectfully submitted,

March 10, 1998
(Date)

By: Kathryn Doyle Leary
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[ ] Additional inventors are being named on separately numbered sheets attached hereto.

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COMPOSITIONS AND METHODS FOR TREATMENT OF ASTHMA

GOVERNMENT SUPPORT

This invention was made in part using funds obtained from the U.S. Government (National Heart, Lung and Blood Institute Grant No. HL-31467) and the U.S. Government may therefore have certain rights in the invention.

FIELD OF THE INVENTION

The field of the invention is asthma therapy.

BACKGROUND OF THE INVENTION

Bronchial asthma in mammals is characterized by inflammation of the airways, exaggerated airway reactivity to bronchoconstrictor agonists, and attenuated beta-adrenoceptor-mediated airway relaxation (Bai, 1990 Am. Rev. Respir. Dis. 141:552-557; Goldie et al., 1986, Br. J. Clin. Pharmacol. 22:669-676; McFadden et al., 1994, Am. J. Respir. Crit. Care Med. 150:523-526). In humans with atopic asthma, mast cell activation has been implicated in mediating the immediate bronchoconstrictor response which acutely follows antigen inhalation. This response is a process which involves IgE-mediated activation of the high affinity IgE receptor (FcεRI), leading to cellular degranulation and the release of various mast cell-derived mediators including histamine, eicosanoids, and specific cytokines (Metzger, 1992, Immunol. Rev. 125:37-48; Beaven et al., 1993, Immunol. Today 14:222-226; Galli, 1993, N. Engl. J. Med. 328:257-265). The identification of Fc receptors on other cell types in the lung (e.g., mononuclear cells, eosinophils, and dendritic cells) suggests that, apart from mast cells per se, these other cell types may also serve to propagate the pro-inflammatory allergic pulmonary response, most likely via the orchestrated extended release of various cytokines (Walker et al., 1992, Am. Rev. Respir. Dis.

146:109-115; Watson et al., 1993, Am. J. Respir. Cell Mol. Biol. 8:365-369; Capron et al., 1984, J. Immunol. 132:462-468; Beasley et al., 1989, Am. Rev. Respir. Dis. 139:806-817; Litchfield et al., 1992, J. Asthma 29:181-191; Barnes et al., 1988, Pharmacol. Rev. 40:49-84; Borish et al., 1991, J. Immunol. 146:63-67. It is believed that immune complex/Fc receptor interactions expressed by these cells, i.e., mononuclear cells, eosinophils, and dendritic cells, potentially underlie the progression of the airway inflammatory and bronchoconstrictor responses in asthma, wherein the immediate bronchoconstriction accompanying antigen exposure is followed by the development of the late phase asthmatic response involving various proinflammatory cells. Indeed, recent studies have demonstrated that expression of the inducible form of the low affinity IgE receptor (FcεRII or CD23) is upregulated on monocytes and alveolar macrophages (Williams et al., 1992, J. Immunol.149:2823-2829), as well as on circulating B lymphocytes (Gagro et al., 1993, Int. Arch. Allergy Immunol. 101:203-208; Rabatic et al., 1993, Exp. Immunol. 94:337-340) isolated from atopic asthmatic subjects. Similarly, exposure of asthmatic subjects to allergen and treatment of isolated monocytes with specific cytokines have been shown to up-regulate FcεRII expression on mononuclear phagocytes (Williams et al., 1992, J. Immunol. 149:2823-2829; Joseph et al., 1983, J. Clin. Invest. 71:221-230). These findings suggest that altered Fc receptor expression and action in some cell types may contribute to the overall pro-inflammatory asthmatic response. While it is known that exposure of isolated rabbit and human airway smooth muscle (ASM) to atopic asthmatic serum induces the autocrine release and action of specific cytokines (notably IL-1ß) by the sensitized ASM cells (Hakonarson et al., 1997, J. Clin. Invest. 99:117-124), the mechanism by which this sensitization is mediated has not been disclosed.

Current treatment options for asthma include medications that control the airway inflammatory component of the disease, e.g., primarily corticosteroids, sodium cromolyn, methylxanthines, leukotriene modifiers) and rapid relief medications that counteract bronchospasm, e.g., primarily beta-adrenergic agents.

There are several disadvantages to using these medications as follows: There is a potential lack of effective sustained action; there are side effects associated with prolonged use of these medications, particularly in the case of corticosteroids and beta-adrenergic agents; there is a progressive loss of sensitivity to these treatments after prolonged use; there is limited efficacy of any of these agents in severe cases of asthma; these agents are non-selective, i.e., they do not specifically target the lung, therefore, side-effects affecting other organs are a potential risk. Furthermore, there are data which document an increased risk of dying from bronchial asthma following prolonged treatment of asthma using long-acting beta-adrenergic agents such as fenoterol (Pearce et al., 1990, Thorax 45:170-175; Spitzer et al., 1992, New Engl. J. Med. 326:560-561).

Approximately fifteen million individuals in the U.S. have asthma and the disease is the cause of more than five thousand deaths annually in the U.S. I children, asthma represents the most prevalent chronic disease, requiring the most frequent use of emergency room visits and hospitalizations. The overall annual cost for asthma care in the U.S. is estimated to be about none billion dollars. Asthma is the most common cause of school and work absenteeism in the U.S.

There is thus a long felt need for additional and more specific and effective compositions and methods for treatment of asthma which additional compositions and methods overcome the deficiencies of the prior art compositions and methods.

SUMMARY OF THE INVENTION

The invention relates to a method of preventing induction of an asthmatic state in a human patient comprising administering to the human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby preventing induction of the asthmatic state in the human. Preferably, the pharmaceutically acceptable carrier is physiological saline.

In one aspect, the ligand is selected from the group consisting of an isolated protein , an isolated polypeptide and a non-peptide.

In a preferred embodiment, the ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody. Preferably, the anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.

In another aspect, the ligand is administered to the human in the form of an isolated DNA encoding and capable of expressing the ligand. The DNA may be formulated in a viral or a non-viral vector. When the DNA is formulated in a viral vector, the viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus. When the DNA is formulated in a non-viral vector, the non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.

In yet another aspect, the anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.

The invention also relates to a method of treating asthma in a human patient comprising administering to the human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby treating asthma in the human. Preferably, the pharmaceutically acceptable carrier is physiological saline.

In one aspect, the ligand is selected from the group consisting of an isolated protein, an isolated polypeptide and a non-peptide.

In a preferred embodiment, the ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody. Preferably, the anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.

In another aspect, the ligand is administered to the human in the form of an isolated DNA encoding and capable of expressing the ligand. The DNA may be formulated in a viral or a non-viral vector. When the DNA is formulated in a viral vector, the viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus. When the DNA is formulated in a non-viral vector, the non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.

In yet another aspect, the anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.

Also included in the invention is a method of treating a human having asthma comprising administering to the human a pharmaceutically effective amount of an isolated nucleic acid encoding an anti-FcεRII receptor protein ligand, wherein the nucleic acid expresses the ligand in vivo in an amount sufficient to treat the asthma.

In addition, the invention includes a method of identifying an anti-FcεRII receptor protein ligand comprising providing a mixture comprising IgE and a population of cells which express an FcεRII receptor protein, incubating the mixture in the presence or absence of a test ligand, and measuring the level of IgE bound to the FcεRII receptor protein, wherein a lower level of IgE bound to the cells in the presence of the test compound compared with the level of binding of IgE to the cells in the absence of the test compound is an indication that the test compound is an anti-FcεRII receptor protein ligand.

In a preferred embodiment of this aspect of the invention, the cells are airway smooth muscle cells.

The invention also includes a ligand useful for inhibiting binding of IgE to an FcεRII receptor protein identified by a method comprising providing a mixture comprising IgE and a population of cells which express an FcεRII receptor protein, incubating the mixture in the presence or absence of a test ligand, and measuring the

level of IgE bound to the FcεRII receptor protein, wherein a lower level of IgE bound to the cells in the presence of the test compound compared with the level of binding of IgE to the cells in the absence of the test compound is an indication that the test compound is an anti-FcεRII receptor protein ligand.

In addition, the invention includes a method of inhibiting binding of IgE to an FcεRII receptor protein expressed on a cell comprising administering to the cell an anti-FcεRII receptor protein ligand.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a graph depicting a comparison of contractile dose-response relationships to acetylcholine (ACh) in paired control serum-treated (open circles) and atopic asthmatic serum-treated tracheal smooth muscle (TSM) segments in the absence (closed circles) and presence (open squares) of anti-CD23 monoclonal antibody (MAb).

Figure 1B is a graph depicting a comparison of contractile dose-response relationships to augmented constrictor responses (ACR) in control serum-treated (open circles) and atopic asthmatic serum-treated TSM in the absence (closed circles) and presence (open squares) of Staphylococcus protein A (SpA). Data represent mean ±SE values from 6 paired tissue samples.

Figure 2A is a graph depicting a comparison of relaxation dose-relationships to isoproterenol in paired control serum-treated (open circles) and atopic asthmatic serum-treated TSM segments half-maximally contracted with their respective ED_{50} doses of ACh in the absence (filled circles) and presence (open squares) of anti-CD23 MAb.

Figure 2B is a graph depicting a comparison of relaxation responses to isoproterenol in control (open circles) and atopic asthmatic serum-treated TSM in the absence (filled circles) and presence (open squares) of SpA. Data are mean ± SE values from 8 paired tissue samples.

Figure 3 is a graph depicting a comparison of airway constrictor responses to ACh in isolated paired TSM segments in the absence (open circles) and presence (filled circles) of IgE immune complexes (see Methods). Data represent mean ±SE values from 6 paired tissue samples.

Figure 4 is a graph depicting a comparison of airway relaxation responses to isoproterenol in paired TSM segments in the absence (open symbols) and presence (filled symbols) of IgE immune complexes (see Methods). All TSM segments were initially half-maximally contracted with their respective ED_{50} doses of ACh prior to isoproterenol administration. Data represent mean ±SE values from 8 paired tissue samples.

Figure 5A is an image of a gel depicting expression of FcγRIII receptor mRNA using reverse transcriptase polymerase chain reaction (RT-PCR) in rabbit ASM cells following 0, 6 and 24 hours of treatment with 10% control serum and 10% atopic asthmatic sensitizing serum. Expression of α-actin was used to control for loading. cDNA obtained from activated U937 cells (i.e., for FcγRIII) was used as a positive control. The blots were probed with human specific FcγRIII ^{32}P-labeled cDNA probes.

Figure 5B is an image of a gel depicting expression of FcεRII receptor mRNA using RT-PCR in rabbit ASM cells following 0, 6 and 24 hours of treatment with 10% control serum and 10% atopic asthmatic sensitizing serum. Expression of α-actin was used to control for loading. cDNA obtained from the immortalized human B-cell line 8.1.6 (i.e., for FcεRII) was used as a positive control. The blots were probed with human specific FcεRII ^{32}P-labeled cDNA probes.

Figure 6A is an image of a Southern blot probed with full length FcγRIII and a 157-bp RPL7 human cDNA probe. Paired human airway smooth muscle samples were incubated with control (CO) or atopic asthmatic (SE) serum for 24 hours. cDNA was transcribed from total RNA primed with oligo(dT). PCR products were amplified using a human-specific FcγRIII and RPL7 primers, run on 1.2% agarose gels, transferred to a Zeta-probe membrane and then probed.

Figure 6B is an image of a Southern blot probed with full length FcεRII and a 157-bp RPL7 human cDNA probe. Paired human airway smooth muscle samples were incubated with control (CO) or atopic asthmatic (SE) serum for 24 hours. cDNA was transcribed from total RNA primed with oligo(dT). PCR products were amplified using human-specific FcεRII and RPL7 primers, run on 1.2% agarose gels, transferred to a Zeta-probe membrane and then probed.

Figure 7 is an image of a gel depicting a expression of FcεRII mRNA as assessed by RT-PCR in human bronchial smooth muscle tissue following 24 hours of treatment with control (CO) or atopic asthmatic serum in the absence (SE) and presence (SE+) of anti-CD23 MAb. The products of the RT-PCR reactions using 2.5 μg of total RNA and human-specific primers for the FcεRII receptor mRNA are shown. Expression of RPL7 was used to control for gel loading. The blot was probed with the above ^{32}P-labeled human-specific FcεRII and RPL7 cDNA probes.

Figure 8A is a gel depicting of expression of FcεRII as assessed by RT-PCR in rabbit ASM cells following 0 and 24 hr treatment with media alone (control) or with IgE immune complexes for 0, 6, 12 and 24 hours. The products of RT-PCR reactions using 2.5 μg of total RNA and human-specific primers for the FcεRII receptor mRNA are shown. mRNA expression of α-actin is also shown for comparison.

Figure 8B is a graph depicting the corresponding time-dependent changes in the FcεRII/α-actin ratio, obtained from the data presented in Figure 8A, expressed as fold increase above baseline (i.e., time 0) in control (open bars) and IgE immune complex-treated cells (filled bars).

Figure 9 is a series of graphs comprising panels A-D, depicting a flow cytometric analysis of FcγRIII and FcεRII surface expression in rabbit ASM cells. Cells treated for 24 hours with either 10% control serum or 10% atopic asthmatic serum were stained with fluorescein isothiocyanate (FITC)-conjugated human monoclonal antibodies specific for the low-affinity FcγRIII (Panel A) and FcεRII (CD23) (Panel B) receptors. Activated B-cells (8.1.6) were used as a positive control

for the CD23 receptor. The level of non-specific background staining was measured in both the control and atopic asthmatic serum-treated cells by staining with FITC-conjugated isotype control antibodies.

Figure 10 is a series of images, comprising Panels A and B, depicting immunofluoresence staining for CD23 surface receptor protein in control (CO) serum-treated (left panels) and atopic asthmatic serum (SE) sensitized (right panels) rabbit ASM cells (A; magnification x 100) and TSM tissue (B; magnification x 50). $F(ab')_2$-FITC conjugated fragments were used to detect the primary monoclonal anti-CD23 antibody. Isotype negative control antibody was used to control for nonspecific staining (upper panels: A and B).

DETAILED DESCRIPTION

The invention relates to the discovery of the expression of an Fc receptor protein on airway smooth muscle cells, which expression plays a significant role in the development of the asthmatic state in an individual having asthma. While expression of the receptor of the invention on other cell types is known, expression of the receptor on airway smooth muscle cells has been heretofore unknown.

The receptor of the invention is a receptor for IgE termed FcεRII or CD23. This receptor is a low affinity receptor for IgE which has been observed to be expressed in an inducible form on monocytes and lung alveolar macrophages (Williams et al., 1992, J. Immunol. 149:2823-2829; Joseph et al., 1983, J. Clin. Invest. 71:221-230), as well as on circulating B lymphocytes (Gagro et al., 1993, Int. Arch. Allergy Immunol. 101:203-208; Rabatic et al., 1993, Exp. Immunol. 94:337-340), isolated from atopic asthmatic individuals. However, until the present discovery, the expression of this receptor on airway smooth muscle cells has not been known.

In addition to the discovery of the expression of the FcεRII receptor on airway smooth muscle cells, the present invention includes the discovery that antibodies directed against this receptor block the induction of the asthmatic state. Thus, the invention includes a method of preventing induction of the asthmatic state in

a mammal, which method comprises administering to the mammal a pharmaceutically effective amount of an anti-FcεRII receptor ligand. The ligand binds to the FcεRII receptor protein, thereby inhibiting binding of IgE to the FcεRII receptor protein. Inhibition of binding of IgE to the FcεRII receptor protein serves to prevent induction of the asthmatic state.

The invention also includes a method of treating asthma in a mammal, which method comprises administering to the mammal a pharmaceutically effective amount of an anti-FcεRII receptor ligand. The ligand binds to the FcεRII receptor protein, thereby inhibiting binding of IgE to the FcεRII receptor protein. Inhibition of binding of IgE to the FcεRII receptor protein serves to diminish or ablate the asthmatic state in the mammal thereby treating asthma in the mammal. Preferably, the mammal is a human.

There are two types of FcεRII receptor proteins, FcεRIIa and FcεRIIb, which differ in only six to seven N-terminal amino acids. Both receptor proteins are derived from the same gene through the use of different promoters which control expression of separate first exons of the protein, which exons are spliced to a common mRNA sequence (Delespease et al., 1992, Immunol. Rev. 125:78-97). The invention should be construed to include both receptor proteins.

By the term "asthmatic state" as used herein, is meant the proasthmatic phenotype which is observed in airway smooth muscle cells. This phenotype is characterized by increased contraction and decreased relaxation of the airway tissue when it has been exposed to high IgE-containing atopic asthmatic serum or exogenous IgE, compared with airway tissue which has not been exposed to IgE.

By the term "treating asthma" is meant curing asthma, causing the symptoms of asthma to diminish, ablating or otherwise alleviating the disease.

The invention should be construed to include any ligand that is currently either known or is heretofore unknown, which when bound to an FcεRII receptor protein on an airway smooth muscle cell of a mammal serves to alleviate an asthmatic state in the mammal.

By the term "ligand" as used herein, is meant any natural or synthetic composition or compound which is capable of specifically binding to its cognate receptor protein, and when so bound, prevents binding of IgE to the cognate receptor protein, such that an asthmatic state is prevented or diminished. By way of example, an antibody which specifically binds to an FcεRII receptor protein on an airway smooth muscle cell and inhibits binding of IgE thereto, is termed an "anti-FcεRII receptor protein ligand." In this context, the FcεRII receptor protein is the "cognate receptor protein" for the ligand.

By the term "anti-FcεRII receptor protein ligand" as used herein, is meant any natural or synthetic composition or compound which is capable of binding to an FcεRII receptor protein on an airway smooth muscle cell, which binding prevents binding of IgE to the cognate FcεRII receptor protein.

Preferably, the anti-FcεRII receptor protein ligand is an antibody.

The invention should not be construed to be limited to the specific ligands and respective cognate receptor proteins disclosed in the Examples presented herein. Rather, the invention should be construed to include any presently known or heretofore unknown ligands which have the effect of inhibiting binding of an IgE molecule to an FcεRII receptor protein on an airway smooth muscle cell. It is a simple matter, upon reading the present disclosure, to use the IgE binding inhibition assays described in the Examples to identify additional ligands which bind to an FcεRII receptor protein and inhibit the binding of IgE thereto. Thus, while preferred ligands for use in the methods of the invention are antibodies directed to an FcεRII receptor protein, yet other useful ligands may be identified using the protocols described herein. The most preferred ligand for use in the methods of the invention is an antibody which is an anti-FcεRII receptor protein ligand.

To identify a ligand capable of binding to an FcεRII receptor protein expressed on an airway smooth muscle cell and inhibiting binding of IgE thereto, a mixture comprising a population of cells, for example, airway smooth muscle cells which express an FcεRII receptor protein, and IgE is incubated in the presence or

absence of a test ligand. Binding of IgE to the cells is then assessed. The level of binding of IgE to cells incubated in the presence of the test compound is compared with the level of binding of IgE to cells in the absence of the test compound. A lower level of IgE binding to cells in the presence of the test compound compared with the level of binding of IgE to the cells in the absence of the test compound is an indication that the test compound is an anti-FcεRII receptor protein ligand. Additional assays which definitively establish that the test compound is capable of binding to an FcεRII receptor protein and preventing the binding of IgE thereto, may then be conducted following the protocols provided herein in the Examples section.

The ligand for use in the method of the invention may be any natural or synthetic composition or compound which when bound to its cognate receptor protein, effects the inhibition of binding of IgE to the cognate receptor protein. Thus, the ligand may be a protein, a peptide or a small molecule. The ligand may be administered to a cell as is, that is, as an isolated protein, an isolated peptide, a small molecule, or it may be administered to the cell in the form of an isolated nucleic acid sequence encoding the ligand.

By the term "isolated nucleic acid", as used herein, refers to a nucleic acid sequence, a DNA or an RNA or fragment thereof which has been separated from the sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *e.g.*, the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, *e.g.*, RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g, as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of

other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences.

In other related aspects, the invention includes vectors which contain such isolated nucleic acid and which are preferably capable of directing expression of the protein encoded by the nucleic acid in a vector-containing cell; and cells containing such vectors, either eukaryotic cells or prokaryotic cells, preferably eukaryotic cells.

The isolated nucleic acid of the invention should be construed to include an RNA or a DNA sequence encoding a ligand of the invention, and any modified forms thereof, including chemical modifications of the DNA or RNA which render the nucleotide sequence more stable when it is cell free or when it is associated with a cell. Chemical modifications of nucleotides may also be used to enhance the efficiency with which a nucleotide sequence is taken up by a cell or the efficiency with which it is expressed in a cell. Any and all combinations of modifications of the nucleotide sequences are contemplated in the present invention.

By the terms "isolated peptide" or "isolated protein," as used herein, is meant a peptide or protein which has been substantially separated from the components, e.g,. DNA, RNA, other proteins and peptides, carbohydrates and lipids, which naturally accompany the protein or peptide in the cell. The terms isolated peptide and protein may be construed to include a peptide or protein which is expressed and/or secreted from a cell comprising an isolated nucleic acid.

The present invention also provides for analogs of proteins or peptides which comprise a ligand as defined herein. Analogs may differ from naturally occurring proteins or peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both. For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function. Conservative amino acid substitutions typically include substitutions within the following groups:

glycine, alanine;

valine, isoleucine, leucine;

aspartic acid, glutamic acid;

asparagine, glutamine;

serine, threonine;

lysine, arginine;

phenylalanine, tyrosine.

Modifications (which do not normally alter primary sequence) include *in vivo*, or *in vitro* chemical derivatization of polypeptides, *e.g.*, acetylation, or carboxylation. Also included are modifications of glycosylation, *e.g.*, those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; *e.g.*, by exposing the polypeptide to enzymes which affect glycosylation, *e.g.*, mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated amino acid residues, *e.g.*, phosphotyrosine, phosphoserine, or phosphothreonine.

Also included are polypeptides which have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. Analogs of such polypeptides include those containing residues other than naturally occurring L-amino acids, *e.g.*, D-amino acids or non-naturally occurring synthetic amino acids. The peptides of the invention are not limited to products of any of the specific exemplary processes listed herein.

Desirable isolated protein or isolated peptide ligands include antibodies which bind to the desired cognate receptor protein. The antibody may be any type of antibody including, but not limited to, a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody, a humanized antibody, and the like. Other anti-FcεRII receptor protein ligands include proteins which are not antibodies, such as

Antibody technology is described in Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY). Polyclonal antibodies directed against an FcεRII receptor protein may be made by immunizing any suitable animal

and obtaining immune serum from the animal at selected intervals following immunization.

Monoclonal antibodies directed against full length or peptide fragments of an FcεRII receptor protein may be prepared using any well known monoclonal antibody preparation procedures, such as those described, for example, in Harlow et al. (supra). Quantities of the desired peptide may also be synthesized using chemical synthesis technology. Alternatively, DNA encoding the desired peptide may be cloned and expressed from an appropriate promoter/regulatory sequence in cells which are suitable for the generation of large quantities of peptide. Monoclonal antibodies directed against the peptide are generated from mice immunized with the peptide using standard procedures as referenced herein.

Nucleic acid encoding the monoclonal antibody obtained using the procedures described herein may be cloned and sequenced using technology which is available in the art, and is described, for example, in Wright et al. (1992, Critical Rev. in Immunol. 12(3,4):125-168) and the references cited therein. Further, the antibody may be "humanized" using the technology described in Wright et al., (*supra*) and in the references cited therein.

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells, *e.g.*, the hybridoma, which express the desired protein to be expressed on the phage surface, *e.g.*, the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies immunoglobulin fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying immunoglobulin genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook et al. (1989, Molecular Cloning. A Laboratory Manual, Cold Spring Harbor, NY) and in Ausubel et al. (Ausubel et al., 1993, Current Protocols in Molecular Biology, Green & Wiley, New York).

Bacteriophage which encode the desired antibody, may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, *e.g.*, the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific antibody are incubated in the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell. Such panning techniques are well known in the art and are described for example, in Wright et al., (supra).

Processes such as those described above, have been developed for the production of human antibodies using M13 bacteriophage display (Burton et al., 1994, Adv. Immunol. 57:191-280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express human Fab fragments on their surface. Phage which display the antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells which express human immunoglobulin.

The procedures just presented describe the generation of phage which encode the Fab portion of an antibody molecule. However, the invention should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFv/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light chain, that is, they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage libraries

comprising scFv DNA may be generated following the procedures described in Marks et al. (1991, J. Mol. Biol. 222:581-597). Panning of phage so generated for the isolation of a desired antibody is conducted in a manner similar to that described for phage libraries comprising Fab DNA.

The invention should also be construed to include synthetic phage display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities (Barbas, 1995, Nature Medicine 1:837-839; de Kruif et al. 1995, J. Mol. Biol. 248:97-105).

By the term "synthetic antibody" as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

The invention thus includes an isolated DNA encoding an anti-FcεRII receptor protein ligand or DNA encoding a portion of the ligand, which when the ligand is, for example, an antibody, the antibody is itself specific for its cognate receptor protein, or for fragments thereof.

To isolate DNA encoding an antibody, for example, DNA is extracted from antibody expressing phage obtained as described herein. Such extraction techniques are well known in the art and are described, for example, in Sambrook et al. (supra) and in Ausubel et al. (supra).

By the term "scFv/phage" are used herein, is meant a phage particle which expresses the Fv portion of an antibody as a single chain.

The invention should be construed to include other anti-FcεRII receptor protein-binding ligands which are either known or are heretofore unknown, which are,

or will be designed to bind to an FcεRII receptor protein and which are useful for treating or preventing the asthmatic state in an individual.

Another form of ligand includes a nucleic acid sequence which encodes the anti-FcεRII receptor protein ligand and which is associated with promoter/regulatory sequences which can direct expression of the anti-FcεRII receptor protein ligand *in vivo*. For a discussion of this technology, see, for example, Cohen (1993, Science 259:1691-1692), Fynan et al. (1993, Proc. Natl. Acad. Sci. 90:11478-11482) and Wolff et al. (1991, Biotechniques 11:474-485) which describe similar the use of naked DNA as a therapeutic agent. For example, a plasmid containing suitable promoter/regulatory sequences operably linked to a DNA sequence encoding an anti-FcεRII receptor protein ligand, may be directly administered to a patient using the technology described in the aforementioned references.

As used herein, the term "promoter/regulatory sequence" means a DNA sequence which is required for tissue-specific, organ specific, or other specific (such as inducible, etc) expression of a gene operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene in the desired specific manner.

By describing two nucleic acid sequences as "operably linked" as used herein, is meant that a single-stranded or double-stranded nucleic acid moiety comprises each of the two nucleic acid sequences and that the two sequences are arranged within the nucleic acid moiety in such a manner that at least one of the two nucleic acid sequences is able to exert a physiological effect by which it is characterized upon the other.

Alternatively, the promoter/enhancer sequence operably linked to DNA encoding the anti-FcεRII receptor protein ligand may be contained within a vector, which vector is administered to the patient. The vector may be a viral vector which is suitable as a delivery vehicle for delivery of the anti-FcεRII receptor protein ligand DNA to the patient, or the vector may be a non-viral vector which is suitable for the

same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in (Ma et al., 1997, Proc. Natl. Acad. Sci. USA 94:12744-12746). Examples of viral vectors include, but are not limited to, a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus, a recombinant avian pox virus, and the like (Cranage et al., 1986, EMBO J. 5:3057-3063; International Patent Application No. WO94/17810, published August 18, 1994; International Patent Application No. WO94/23744, published October 27, 1994). Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA, and the like.

The identity, selection and means for obtaining a desired anti-FcεRII receptor protein ligand useful for treatment or prevention of asthma may be performed by the skilled artisan using conventional technology when in possession of the present invention. For example, as described in the Examples presented herein, there are a variety of anti-FcεRII monoclonal antibodies which are available that are likely anti-FcεRII receptor protein ligands suitable for use in the methods of the invention.

Other anti-FcεRII receptor protein ligands may include, but are not limited to, isolated proteins and isolated polypeptides and isolated nucleic acid sequences encoding the same. Isolated proteins and peptides having anti-FcεRII receptor protein ligand activity and isolated nucleic acids encoding the same, may be chemically synthesized by conventional methods known in the art, or they may be purchased for a commercial source if available. In one embodiment of the invention, the anti-FcεRII receptor protein ligand, being a protein, a peptide or a nucleic acid, may be produced using recombinant techniques *in vitro* in sufficiently large quantities for use in a therapeutic composition for use in treating or preventing asthma. In addition, a recombinant virus vector comprising DNA encoding the desired anti-FcεRII receptor protein ligand may be prepared using conventional recombinant DNA technology procedures.

The ligand useful in the methods of the invention may be a small molecule, a non-peptide, a peptidometic, and the like, which ligand binds to an FcεRII receptor protein thereby inhibiting binding of IgE to the receptor protein. Once in possession of the present invention, it is within the skill of the ordinary artisan to identify the contact points between the ligand and the cognate receptor protein, which contact points are essential for binding of these molecules together to inhibit binding of IgE to the receptor protein. Thus, it is also within the skill of the artisan to design specific peptidometics which bind to FcεRII receptor protein. The invention should be construed to include such peptidometics. The technology of the development of peptidometics is described, for example, in PCT/US93/01201 and U.S. Patent No. 5,334,702.

The anti-FcεRII receptor protein ligand of the invention may be formulated in a pharmaceutical composition which is suitable for administration of the ligand to a human patient. It will be appreciated that the precise formulation and dosage amounts will vary depending upon any number of factors, including, but not limited to, the type and severity of the disease to be treated, the route of administration, the age and overall health of the individual, the nature of the ligand, etc. However, the preparation of a pharmaceutically acceptable composition having an appropriate pH, isotonicity, stability and other characteristics is within the skill of the art. Pharmaceutical compositions are described in the art, for example, in Remington's Pharmaceutical Sciences (Genaro ed., 1985, Mack Publishing Co., Easton, PA).

As used herein, the term "pharmaceutically-acceptable carrier" means a chemical composition with which an appropriate anti-FcεRII receptor protein ligand, may be combined and which, following the combination, can be used to administer the ligand to a patient.

The amount of the anti-FcεRII receptor protein ligand composition administered, whether it is administered as protein or as nucleic acid, is sufficient to prevent, diminish or alleviate the asthmatic state. The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between

about 1 ng/kg and about 100 mg/kg of patient body weight. Suitable amounts of the anti-FcεRII receptor protein ligand for administration include doses which are high enough to have the desired effect without concomitant adverse effects. When the anti-FcεRII receptor protein ligand is a protein or peptide, a preferred dosage range is from about 10 to about 1000 μg of protein or peptide per kg of patient body weight. When the anti-FcεRII receptor protein ligand is administered in the form of DNA encoding the same contained within a recombinant virus vector, a dosage of between about 10^2 and about 10^{11} plaque forming units of virus per kg of patient body weight may be used. When naked DNA encoding the anti-FcεRII receptor protein ligand is to be administered as the pharmaceutical composition, a dosage of between about 10 μg about several mg of DNA per kg of patient body weight may be used.

In the practice of the methods of the invention, a composition containing an anti-FcεRII receptor protein ligand is administered to a patient in a sufficient amount to prevent, diminish or alleviate an asthmatic state in the individual. Patients to be treated include children and adults who have atopic (allergic) asthma. This constitutes the vast majority of asthmatic individuals.

The frequency of administration of an anti-FcεRII receptor protein ligand to a patient will also vary depending on several factors including, but not limited to, the type and severity of the asthma to be treated, the route of administration, the age and overall health of the individual, the nature of the anti-FcεRII receptor protein ligand, etc. It is contemplated that the frequency of administration of the anti-FcεRII receptor protein ligand to the patient may vary from about once every few months to about once a month, to about once a week, to about once per day, to about several times daily.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in parenteral, oral solid and liquid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. In addition to the appropriate anti-FcεRII receptor protein ligand, these pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients

known to enhance and facilitate drug administration. Thus such compositions may optionally contain other components, such as adjuvants, e.g., aqueous suspensions of aluminum and magnesium hydroxides, and/or other pharmaceutically acceptable carriers, such as saline. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer the appropriate anti-FcεRII receptor protein ligand to a patient according to the methods of the invention.

Preferably, the composition of the invention is administered to the human by a lung inhalation route, i.e., via a nebulizer or other lung inhalation device.

An anti-FcεRII receptor protein ligand may be administered in conjunction with other compounds which are used to treat asthma. Such compounds include, but are not limited to, corticosteroids, sodium cromolyn, methylxanthines, leukotriene modifiers), anti-cholinergic agents,and rapid relief medications that counteract bronchospasm, e.g., primarily beta-adrenergic agents. The choice of which additional compound to administer will vary depending upon any number of the same types of factors that govern the selection of dosage and administration frequency of the anti-FcεRII receptor protein ligand. Selection of these types of compounds for use in conjunction with an anti-FcεRII receptor protein ligand for practice of the method of the invention is well within the skill of those in the art.

The invention will be further described by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Examples

The experiments presented were designed to examine whether ASM cells have the capacity to intrinsically express Fc receptors. The experiments were also designed to examine whether the expression and activation of these Fc receptors

is altered in the atopic asthmatic sensitized state, which alteration may contribute to changes in agonist responsiveness of the ASM tissue. The results demonstrate that a) enhanced constrictor and attenuated relaxation responsiveness induced in ASM passively sensitized with human atopic asthmatic serum are prevented by depleting the sensitizing serum of its immune complexes or by blockade of FcεRII activation with an anti-CD23 blocking antibody; b) exposure of ASM cells and tissue to atopic asthmatic serum induces autologously upregulated mRNA and cell surface expression of FcεRII receptors by the ASM and; c) the latter induced upregulated expression of the FcεRII receptor in the atopic sensitized state is ablated by an anti-CD23 blocking antibody. Taken together, these observations provide new evidence that airway smooth muscle cells intrinsically express Fc receptors and that the induced altered responsiveness of atopic asthmatic sensitized airway smooth muscle is largely attributed to its autologously upregulated expression and activation of the FcεRII receptor subtype. Notwithstanding the well-established contribution of airway infiltrating inflammatory cells in the pathogenesis of atopic asthma (Beasley et al., 1989, Am. Rev. Respir. Dis. 139:806-817; Litchfield et al., 1992, J. Asthma 29:181-191; Barnes et al., 1988, Pharmacol. Rev. 40:49-84), the present novel findings identify a critical role for the airway smooth muscle itself in autologously regulating IgE/CD23-coupled changes in airway reactivity which characterize the asthmatic state.

The data may be summarized as follows. To elucidate the role of immunoglobulin (Ig) E-dependent mechanisms in inducing altered airway responsiveness in the atopic asthmatic state, the expression and actions of Fc receptor activation were examined in isolated rabbit tracheal smooth muscle (TSM) tissue and cultured cells passively sensitized with sera from atopic asthmatic patients or nonatopic/nonasthmatic (control) subjects. Relative to control tissues, the atopic asthmatic-sensitized TSM exhibited significantly increased maximal isometric contractility to acetylcholine ($p<0.01$) and attenuated maximal relaxation responses and sensitivity (i.e., -log ED_{50}) to isoproterenol ($p<0.005$). These changes in agonist

responsiveness in atopic sensitized TSM were ablated by pretreating the tissues with a monoclonal blocking antibody (MAb) to the low affinity receptor for IgE, FcεRII (i.e., CD23) or by depleting the sensitizing serum of its immune complexes. Moreover, in complimentary experiments, exogenous administration of IgE immune complexes to naive TSM produced changes in agonist responsiveness which were qualitatively similar to those obtained in the atopic asthmatic sensitized state. Additional experiments established that, in contrast to their respective controls, atopic asthmatic serum-sensitized human and rabbit TSM tissue and cultured cells exhibited markedly induced mRNA and cell surface expression of FcεRII, whereas constitutive expression of the IgG receptor subtype, FcγRIII, was unaltered. Moreover, the upregulated mRNA expression of FcεRII observed following exposure of TSM to atopic asthmatic serum or to exogenously administered IgE immune complexes was significantly inhibited by pretreating the tissues or cells with anti-CD23 MAb. Collectively, these data provide new evidence demonstrating that the altered agonist responsiveness in atopic asthmatic sensitized airway smooth muscle is largely attributed to IgE-mediated induction of the autologous expression and activation of FcεRII receptors in the airway smooth muscle itself.

The Materials and Methods used in the experiments presented herein are now described.

Animals

Thirty four adult New Zealand White rabbits were used in the present study which was approved by the Biosafety and Animal Research Committee of the Joseph Stokes Research Institute at The Children's Hospital of Philadelphia. The animals exhibited no signs of respiratory disease for several weeks before the study.

Preparation and sensitization of airway smooth muscle tissue

Rabbits were anesthetized with xylazine (10 mg/kg) and ketamine (50 mg/kg) and were subsequently sacrificed using an overdose of pentobarbital (130 mg/kg). The tracheae of the rabbits were removed via open thoracotomy, the tracheae were cleared of loose connective tissue and were divided into eight ring segments of 6-

8 mm length. Each segment referred to herein as a tracheal smooth muscle (TSM) segment was incubated for 24 hours at room temperature in either of the following: 1) human serum containing immunoglobulin (Ig) E at greater than 1000 IU/ml which was obtained from allergic patients having moderate to severe asthma and a 4-5/6+ radioallergosorbent test (RAST) positive (having a specific IgE concentration of more than 17.5 Phadebas RAST units (PRU)/ml) to *Dermatophagoides pteronyssimus*, *Dermatophagoides farinae* and ragweed, and a positive skin test to these antigens; or 2) human serum obtained from non-atopic, non-asthmatic individuals having normal serum IgE levels (i.e., < 70 IU/ml) and negative skin test reactivity to *Dermatophagoides pteronyssimus*, *Dermatophagoides farinae* and ragweed (Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). In parallel experiments, TSM segments were incubated in either control serum or in atopic asthmatic serum that was: a) depleted of its immunoglobulin complexes by pretreatment with sepharose Staphylococcus protein A as described (Kessler, 1975, J. Immunol. 115:1617-1624); b) co-incubated with an anti-CD23 (40 μg/ml) monoclonal blocking antibody; or c) with an anti-CD16 (2 μg/ml) monoclonal blocking antibody. The TSM segments were treated with their respective anti-CD23 or anti-CD16 antibodies in Dulbecco's modified Eagle's medium for 1 hour prior to exposure to the atopic asthmatic serum. In extended experiments, paired TSM segments were incubated for 24 hours in Dulbecco's modified Eagle's medium, as previously described (Hakonarson et al., 1996, J. Clin. Invest. 97:2593-2600), containing either: a) human IgE (final bath concentration (FBC): 15 μg/ml); or b) human IgE-goat-anti-human IgE immune complexes (FBC: 15:5 μg/ml of IgE/anti-IgE in the final organ bath mixture). Tissues incubated in medium alone served as controls. The concentrations of immune complexes used for the TSM incubations were based on the results obtained in pilot studies which were designed to determine the concentration and ratio of immune complexes that induced the greatest acute contractile effect in isolated sensitized TSM segments. The serum was aerated with a continuous supplemental O_2 mixture (95% O_2/5% CO_2) during the incubation phase.

In comparable experiments, the passive sensitization protocol described above was also conducted on human tracheal smooth muscle which was isolated 1 hour post-mortem from a 53 year-old male who died from a central nervous system complication of immune thrombocytopenia and had no evidence of lung disease. The passive sensitization protocol was also conducted on a surgically resected human bronchial smooth muscle segment obtained from a 72 year-old female with peripheral lung carcinoma. Microscopically normal airway smooth muscle was carefully cleaned of loose connective tissue and epithelium and divided into 4-5 mm long strips. As above, each alternate adjacent strip was incubated in either control or atopic asthmatic serum in the absence or presence of an anti-CD23 antibody and was subsequently examined for Fc receptor expression (see below).

Preparation and sensitization of airway smooth muscle cells

Airway smooth muscle (ASM) cells were cultured according to described protocols. These cells have been characterized in detail with respect to their distinguishing morphological, histological, and immunological features. The cell isolation and subcultivation procedures, and the characterization of the cells is described in Noveral et al. (1992, Am. J. Physiol. (Lung Cell. Mol. Physiol.) 263:L555-L561). Briefly, ASM cells were isolated from epithelium-denuded trachealis muscle obtained from adult New Zealand White rabbits. Following digestion in F-12 culture medium containing 30 mg/ml protease, 55 mg/ml type IV collagenase, and 100 mg/ml trypsin inhibitor, the dissociated cells were centrifuged and resuspended in F-12 containing 10% fetal bovine serum (FBS) and 100 μg/ml of gentamicin sulfate. The cells were then inoculated in 100 mm tissue culture dishes and, after 4 weeks, the cells had sufficiently proliferated to permit routine subcultivations. At weekly intervals, the subcultivated cells were suspended and then inoculated at a density of 1 x 10^4 cells/cm^2 in 75 cm^2 tissue culture flasks containing F-12 supplemented with 20% FBS and were then incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air. Routine tests for mycoplasma contamination were negative. For the sensitization protocol, cells initially seeded at 1 x 10^4 cells/cm^2 in 75-

cm^2 tissue culture flasks were grown to confluence in Ham's F-12 medium containing 20% FBS. The original culture medium was then replaced with Ham's F-12 for 24 hours and was subsequently changed to F-12 containing either of the following: 1) 10% human serum containing immunoglobulin (IgE) E at greater than 1000 IU/ml obtained from allergic patients with moderate to severe asthma (as above); 2) 10% human serum obtained from non-atopic non-asthmatic (control) individuals (Hakonarson et al., 1996, J. Clin. Invest. 97:2593-2600); 3) IgE immune complexes (15:5 µg/ml), or; 4) F-12 alone, for the various time points.

Pharmacodynamic studies

Following incubation of the tissue preparations obtained as described herein, each rabbit airway segment was suspended longitudinally between stainless steel triangular supports in siliconized Harvard 20 ml organ baths. The lower support was secured to the base of the organ bath, and the upper support was attached via a gold chain to a Grass FT.03C force transducer from which isometric tension was continuously displayed on a multichannel recorder. Care was taken to place the membranous portion of the trachea between the supports in order to maximize the recorded tension generated by the contracting trachealis muscle. The tissues were bathed in modified Krebs-Ringer solution containing 125 mM NaCl, 14 mM $NaHCO_3$, 4 mM KCl, 2.25 mM $CaCl_2.H_2O$, 1.46 $MgSO_4.H_2O$, 1.2 mM $NaH_2PO_4.H_2O$ and 11 mM glucose. The baths were aerated with 5% CO_2 in oxygen; a pH of 7.35-7.40 was maintained, and the organ bath temperature was held at 37°C. Passive resting tension of each TSM segment was set at 2.0 g after each tissue had been passively stretched to a tension of 8 g in order to optimize the resting length of each segment as described (Tanaka et al., 1990, J. Clin. Invest. 85:345-350). The tissues were allowed to equilibrate in the organ baths for 45 minutes, at which time each tissue was primed with a 1 minute exposure to 10^{-4} M acetylcholine (ACh). Cholinergic contractility was subsequently assessed in the TSM segments by cumulative administration of ACh in final bath concentrations ranging from 10^{-10}-10^{-3} M. Thereafter, in separate studies, relaxation dose-response curves to isoproterenol (10^{-10}-10^{-4} M) were conducted in

tissues half-maximally contracted with ACh. The relaxant responses to isoproterenol were analyzed in terms of % maximal relaxation (Rmax) from the active cholinergic contraction, and sensitivity to the relaxing agent was determined as the negative logarithm of the dose of the relaxing agent producing 50% of Rmax (pD_{50}) (i.e., geometric mean ED_{50} value).

Characterization of Fc receptor mRNA by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from sensitized and control ASM cells and tissue using the modified acid guanidinium thiocyanate phenol-chloroform extraction method (Chomczynski et al., 1987, Anal. Biochem. 162:156-159) which includes proteinase K (in 0.5% SDS) digestion of protein in the initial RNA pellet. The concentration of each RNA sample was then determined spectrophotometrically. This procedure consistently yielded 20-25 µg of intact RNA per each T-75 flask of ASM cells.

To analyze mRNA expression specific for the FcγRIA, R-IIA, -IIC, -IIB, -RIII, and FcεRII receptor subtypes, an RT-PCR protocol and human-specific primers for these Fc receptor subtypes was used. cDNA was synthesized using 2.5 µg of total RNA isolated from the cell and tissue samples following 0, 6 and 24 hours of treatment with either 10% control or 10% atopic asthmatic human serum in the presence or absence of an anti-CD23 blocking antibody, and using RNA from cells treated with IgE immune complexes or F-12 serum-free medium alone. cDNA generated using RNA obtained from immortalized U937 cells and the B cell line 8.1.6, were used as positive controls for FcγRIII and FcεRII mRNA expression, respectively. The cDNA was primed with oligo (dT)12-18 and with random hexamer nucleotides (N6) in the absence of signal using the former method. Two µl of cDNA was used in each PCR reaction. The Fc receptor primers used in the PCR assays were based on the published sequences of the human Fcγ -RI, -RII, -RIII and FcεRII genes (Capel et al., 1994, Immunometh. 4:25-34; Kikutani et al., 1986, Cell 47:657-665) and included the following primer sets:

Fcγ RI: 5' Primer: 5'-ATGTGGTTCTTGACAACTCTGCTC 3'
(SEQ ID NO:1)
3' Primer: 5'-ATGTCTGTCTTCTTGAAGGCTGGA-3'
(SEQ ID NO:2) (product is 1,038 bp)

Fcγ RIIA,C: 5' Primer: 5'-GACTCCATTCAGTGGTTCCA-3'
(SEQID NO:3)
3' Primer: 5'-GTCAGCTGTTTCATAGTCATTG-3'
(SQ ID NO:4) (product is 644 bp)

Fcγ RIIB: 5' Primer: 5'-GACTCCATTCAGTGGTTCCA-3
(SEQ ID NO:5)
3' Primer: 5'-CCCAACTTTGTCAGCCTCATC- 3'
(SEQ ID NO:6) (product is 618 bp)

Fcγ RIII: 5' Primer: 5'-AAGATCTCCCAAAGGCTGTG-3'
(SEQ ID NO:7)
3' Primer: 5'-ATGGACTTCTAGCTGCACCG-3'
(SEQ ID NO:8) (product is 254 bp)

FcεRII: 5' Primer: 5'-CGTCTCTCAAGTTTCCAAG-3'
(SEQ ID NO:9)
3' Primer: 5'-GCACTTCCGTTGGGAATTTG-3'
(SEQ ID NO:10) (product is 333 bp)

Rabbit specific α-actin primers, 5'-CGACATCAAGGAGAAGCTG-3' (SEQ ID NO:11) and 5'-CTAGAAGCATTTGCGGTGC-3' (SEQ ID NO:12) (19 mers), and human specific ribosomal protein L7 (RPL7) primers 5'-AAGAGGCTCTCATTTTCCTGGCTG-3' (SEQ ID NO:13) (24 mer) and 5'-TCCGTTCCTCCCCATAATGTTCC-3' (SEQ ID NO:14) (23 mer), based on the published sequence of the rabbit α-actin (Putney et al., 1983, Nature 302:718-721) and human RPL7 genes (Seshadri et al., 1993, J. Biol. Chem. 268:18474-18480), respectively, were used to assess general transcription levels in each sample. The cycling profile used was as follows: Denaturation: 95° C for 1 minute; annealling: 54°

C for 1 minute; and extension: 72° C for 1-2 minutes using 40 cycles for the Fcγ RI, RII, RIII and Fcε RII genes; and using 22 cycles for the α-actin and RPL7 genes, respectively. The number of cycles was determined to be in the linear range of production of the PCR product. The PCR reactions using the human Fcγ, Fcε, RPL7, and rabbit α-actin primers were performed using equivalent amounts of cDNA prepared from 2.5 μg of total RNA. Equal aliquots of each PCR reaction were then run on a 1.2% agarose gel and were subsequently transferred to a Zeta-probe membrane overnight in 0.4 N NaOH. Following capillary transfer, the DNA was immobilized by UV-crosslinking using a Stratalinker UV Crosslinker 2400 at 120,000 microjoules/cm^2 (Stratagene).

Prehybridization in a Techne hybridization oven was conducted for 2-3 hr at 42° C in 50% formaldehyde, 7% (w/v) SDS, 0.25 M NaCl, 0.12 M Na2HPO4 (pH 7.2), and 1 mM EDTA. Hybridization was for 20 hr at 42°C in the same solution. The Fcγ RI, RII, RIII; Fcε RII; RPL7 and α-actin DNA levels were assayed by Southern blot analysis using ^{32}P-labeled probes. The FcγRIII, α-actin and RPL7 probes were prepared by pooling several RT-PCR reactions which used the FcγRIII, α-actin and RPL7 PCR fragments and purifying them from a 1.2% agarose gel using Qiaex II agarose gel extraction kit. The FcγRIII, α-actin and RPL7 cDNA fragments were subsequently sequenced for product confirmation. The other individual human Fcγ and Fcε probes were obtained from the cloned cDNA sequences of these genes. In addition, the 333 bp rabbit ASM CD23 RT-PCR products were also sequenced for product confirmation, and exhibited ~90% homology with the human CD23 B-lymphocyte receptor gene. Washes were as follows: 1 x 15 minutes in 2 X SSC, 0.1% SDS; 1 x 15 minutes in 0.1 X SSC, 0.1% SDS both at room temperature, and 2 x 15 minutes at 50° C in 0.1 X SSC, 0.1% SDS. Southern blots were quantitated by direct measurements of radioactivity in each band using a Phosphoimager (Molecular Dynamics).

Determination of Fc receptor expression in ASM cells and tissue

Fc receptor cell surface expression was examined in cultured rabbit ASM cells using a Coulter EPICS Elite flow cytometer (Coulter EPICS Division, Hialeah, FL) equipped with a 5 watt argon laser operated at 488 nM and 300 mwatt output. Fluorescence signals were accumulated as two parameter fluorescence histograms wherein both % positive cells and mean channel fluorescence were recorded. Cells which were treated for 24 hours with either 10% atopic asthmatic or 10% control human serum were resuspended in buffer, dispersed by passage through a 23 g needle, and then stained with the individual antibodies. Based on the results of the Fc receptor mRNA expression studies, the targeted monoclonal antibodies used included the 3G8-FITC anti-FcγRIII (Medarex, Inc., Annandale, NJ) and the FITC mouse monoclonal antibody to human CD23 (Calteg, San Francisco, CA). The immortalized B-cell line 8.1.6, was used as the positive control for the CD23 receptor expression assay. The cells were also stained with FITC- and PE- conjugated mouse antibodies having the identical isotypes as the Fc receptor monoclonals to measure background fluorescence (i.e., IgG3-FITC as control for anti-CD23; and IgG_2a-PE as control for FcγRIII, respectively). The antibody- stained cells were then evaluated by flow cytometry and were analyzed using the Elite Immuno 4 statistical software (Coulter EPICS Division, Hialeah, FL). Fluorescence intensities were expressed as % positive cells, including mean channel fluorescence.

An immunofluorescence detection assay was also used to examine for FcεRII surface receptor expression in rabbit ASM cells and tissue following 24 hours of treatment with human control vs. atopic asthmatic serum, as described above. The ASM cells were fixed in acetone alone, whereas the tissues were embedded in OCT compound (Miles Laboratories) and frozen in acetone/dry ice. Serial 3-5 μm TSM sections were prepared and mounted on poly-L-lysine coated slides. The specimens were incubated with PBS buffer containing 10% rabbit serum to suppress non-specific staining and were subsequently labeled overnight at 4° C with primary mouse anti-human CD23 (FcεRII) antibody at a 1:250-500 dilution. In control sections, the primary antibody was replaced by immunoglobulins of the same isotype following the

manufacture's protocol (mouse IgG1 negative control). Parallel cell and tissue slides were also stained with an α-actin antibody. After subsequent repeat washing, FITC-labeled $F(ab')_2$ goat anti-mouse IgE (Fcε) fragments was added as the secondary antibody. The mixtures were incubated for 1 hour in 1:500-1000 dilutions in PBS containing 0.5% BSA. After serial washing, the slides were examined using a fluorescent microscope, and quantitative analysis of the protein localization in the cell and tissue sections under the above experimental conditions was performed using the Metamorph Imaging System interfaced with a Nikon Diaphot 300 Image Analyzer that utilizes a Hamamatsu CCD camera.

Statistical analysis

Unless otherwise indicated, results are expressed as mean ±SE. Statistical analysis was performed by means of two-tailed paired Student's t-test. P-values < 0.05 were considered significant.

Reagents

The Fcγ-RI, -RII, -RIII, FcεRII, RPL7 and rabbit α-actin primers were obtained from Integrated DNA Technologies Inc., Coralville, IA. ACh and isoproterenol hydrochloride were obtained from Sigma Chemical, St. Louis, MO. The human IgG, goat-antihuman IgG, human myeloma IgE, and the goat-antihuman IgE antibodies were purchased from Biodesign Int. Kennebunk, ME. The 3G8-FITC anti-FcγRIII and FITC-mouse monoclonal antibody to the human CD23 receptors used in flow cytometric studies were purchased from Medarex, Inc., Annandale, NJ and Calteg, San Francisco, CA, respectively. The anti-CD23 monoclonal blocking antibody (mAb135) is described in Mossalayi et al. (1992, EMBO J. 11:4323-4328). The FcεRII (CD23) antibody and $F(ab')_2$-FITC fragments used in the immunofluoresence studies were purchased from Serotec Ltd., Oxford, UK. The immortalized B-cell line 8.1.6, is described in Weenink et al. (1977, International Immunol. 9(6):889-896). The Fcγ -RI, -RII -RIII and FcεRII cDNA probes are described in McKenzie (1994, Current Opinion in Hematol. 1:45-52). All drug concentrations are expressed as final bath concentrations. Isoproterenol and ACh were made fresh for each experiment, dissolved

in normal saline to prepare 10^{-4} M and 10^{-3} M solutions, respectively. The human tissue was provided by the Cooperative Human Tissue Network which is funded by the National Cancer Institute.

The Results of the experiments presented herein are now described.

Role of Fc receptors in altered responsiveness of atopic asthmatic sensitized airway smooth muscle

Passive sensitization of isolated naive airway smooth muscle (ASM) tissue with human atopic asthmatic serum induces changes in the tissue's agonist-mediated constrictor and relaxant responsiveness that phenotypically resemble the pro-asthmatic state (Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). To examine whether these effects of atopic asthmatic serum are mediated, at least in part, by the presence of elevated levels of IgE in the sensitizing serum, constrictor and relaxation responses were separately examined in TSM segments that were treated with human control or atopic asthmatic serum in the absence and presence of a blockade of specific Fc receptors, or following depletion of immune complexes in the sensitizing serum by treatment with Sepharose Staphylococcus protein A (SpA) (Kessler, 1975, J. Immunol. 115:1617-1624). As shown in Figure 1, relative to tissues incubated with control serum (open circles), the maximal constrictor (Tmax) responses to ACh were significantly enhanced in TSM passively sensitized with atopic asthmatic serum (filled circles). Accordingly, the mean ±SE Tmax values amounted to 121.7±5.3 and 146.0±15.5 g/g TSM wt. in the control and sensitized tissues, respectively ($p < 0.01$). The induced augmented constrictor responses to ACh, however, were largely prevented in atopic serum-sensitized tissues that were pre-treated with an anti-CD23 monoclonal blocking antibody (anti-CD23 MAb) (Figure 1A; open squares) or when the sensitizing serum was depleted of its immune complexes by pre-treatment with SpA (Figure 1B; open squares).

In separate studies, during comparable levels of initial sustained ACh-induced contractions in atopic asthmatic sensitized and control serum-treated airway

segments, averaging 38 and 43% of Tmax, respectively, administration of the beta-adrenergic receptor agonist, isoproterenol, elicited cumulative dose-dependent relaxation of the pre-contracted TSM segments (Figure 2). Relative to control TSM, the maximal relaxation responses (Rmax) and sensitivities (pD_{50}; i.e., -log ED_{50}) to isoproterenol were significantly attenuated in the atopic asthmatic serum-sensitized TSM. Accordingly, the mean Rmax values for isoproterenol amounted to 26.0±5.1 % in the atopic sensitized TSM, compared to 47.0±4.7 % in the control serum-treated TSM ($p<0.005$), with corresponding pD_{50} values averaging 5.82±0.16 and 6.59±0.11 -log M, respectively ($p<0.005$). However, the attenuated isoproterenol-induced relaxation responses were ablated in atopic serum-sensitized TSM that were pretreated with anti-CD23 MAb (Figure 2A), or when the sensitizing serum was initially depleted of its immune complexes with SpA (Figure 2B).

In contrast to the above observations obtained in atopic asthmatic serum-sensitized TSM, in tissues incubated with control serum, neither anti-CD23 MAb nor pretreatment of the control serum with SpA affected the subsequent contractility of the tissue to ACh or relaxation responsiveness to isoproterenol. Moreover, contrasting the above inhibitory effects of anti-CD23 MAb in atopic sensitized TSM, exposure of the sensitized tissues to a monoclonal blocking antibody to the FcγRIII receptor, which is also expressed in ASM (see below), had no appreciable effect on the heightened constrictor responsiveness of the tissue to ACh or attenuated relaxation to isoproterenol.

Effects of IgE and IgE immune complexes on ASM responsiveness.

In light of the above observations suggesting a role for an IgE immune complex/FcεRII receptor interaction in mediating altered ASM responsiveness in the atopic asthmatic sensitized state, the issue of whether the sensitizing effects of the atopic asthmatic serum could be simulated by treatment of naive TSM with either exogenous IgE or IgE immune complexes was examined. Constrictor and relaxant dose-response relationships to ACh and isoproterenol, respectively, were separately compared in TSM exposed for 24 hours to vehicle alone (control) or varying

concentrations of IgE or IgE immune complexes. Relative to their respective controls, the maximal constrictor responses to ACh were significantly ($p < 0.05$) enhanced in tissues treated for 24 hours with an optimal concentration of IgE immune complexes (i.e., 15:5 μg/ml of IgE/anti-IgE), wherein the mean ± Tmax responses amounted to 108.8±10.2 vs 94.2±5.1 g/g TSM wt obtained in the control tissues (Figure 3). Comparably, during similar initial sustained levels of ACh-induced contractions (i.e., ~45% Tmax), the subsequent relaxation responses to cumulative administration of isoproterenol were markedly reduced in the IgE immune complex-treated vs. control tissues (Figures 4), wherein the Rmax responses amounted to 36.8±10.4 vs. 67.8±6.0 %, respectively ($p<0.05$), and the corresponding pD_{50} values averaged 6.70±0.08 and 6.84±0.11 -log M, respectively ($p<0.05$). Qualitatively, similar results were obtained in tissues treated with IgE alone, although the changes in ACh-induced contraction and attenuation of isoproterenol-induced relaxation in these tissues were quantitatively less pronounced than those obtained in IgE immune complex-treated TSM.

Fc receptor expression in atopic asthmatic sensitized airway smooth muscle

In extending the above pharmacodynamic studies, in separate experiments, the issues of whether cultured ASM cells and isolated ASM tissue express Fc receptors and whether the expression of these receptors is modulated in the atopic asthmatic sensitized state was examined. Using RT-PCR and Fc receptor specific primers for the different Fcγ and Fcε receptor subtypes, cDNA was reverse transcribed from total isolated RNA primed with random hexamer primers, and Southern blots were probed with human cDNA probes specific for individual Fcγ and Fcε receptor genes as described in the Methods section. A 415-bp α-actin probe was also used to control for gel loading, and the signals for the Fcγ, Fcε and α-actin PCR products were quantitated on a Phosphorimager.

In contrast to the lack of detectable expression of FcγRI and FcγRII specific mRNA, ASM cells expressed mRNA specific for the FcγRIII receptor (Figure 5A). In parallel with the unaltered constitutive expression of α-actin mRNA, the

FcγRIII signal did not temporally vary in cells treated for up to 24 hours with either control or atopic sensitized serum, and there were no significant differences in FcγRIII mRNA expression between control and sensitized cells. Expression of the high affinity IgE receptor, FcεRI, was also undetectable in the ASM cells. On the other hand, as shown in Figure 5B, expression of mRNA specific for the low affinity IgE receptor, FcεRII (i.e., CD23), was detected in both the control and atopic asthmatic sensitized cells. Moreover, in contrast to control serum-treated ASM cells wherein the mRNA signal did not systematically vary with time, the intensity of the FcεRII mRNA signal progressively increased at 6 and 24 hours in the atopic asthmatic serum-sensitized cells. Thus, rabbit ASM cells expressed mRNA specific for both the low-affinity FcγRIII and FcεRII receptors as verified by the presence of 254 bp (FcγRIII) and 333 bp (FcεRII) cDNA fragments. In contrast to FcγRIII, expression of FcεRII was significantly upregulated at 24 hr (i.e., > 2 fold) following treatment with atopic asthmatic serum.

Similar results were obtained in comparable experiments conducted on isolated human tracheal smooth muscle tissue wherein adjacent alternating sections were exposed for 24 hours to either control or atopic asthmatic serum. Following RNA extraction, PCR products generated using oligo (dT)-primed human cDNA and the above human-specific FcγRIII and FcεRII primers, as well as a primer for the constitutively expressed ribosomal protein, RPL7, were loaded in separate lanes on a 1.2% agarose gel. The Southern blots were then analyzed using ^{32}P-labeled human-specific FcγRIII, FcεRII, and RPL7 (157-bp) probes. As depicted in Figure 6, following 24 hours of incubation with serum, the signals for expression of FcγRIII and RPL7 mRNA were similar in the control (CO) and atopic asthmatic-sensitized (SE) tissue samples (Figure 6A). In contrast, the signal for FcεRII mRNA expression, although detectable in the control samples, was markedly induced in the atopic asthmatic serum-treated tissues (Figure 6B), whereas that of RPL7 was unaffected in these cells. Thus, a marked induction of FcεRII expression was observed in the atopic

asthmatic serum-sensitized sample (i.e., > 7.5 fold), whereas expression of FcγRIII or RPL7 was similar in both samples.

In light of the above observations, together with the preceding pharmacodynamic findings implicating a role for IgE immune complex/CD23 interaction in mediating the changes in agonist responsiveness in the atopic asthmatic sensitized tissues (Figures 1 and 2), the issue of whether the observed upregulation of FcεRII expression in the atopic asthmatic sensitized state was attributable to activation of the FcεRII receptor was examined. As shown in Figure 7, using ASM tissue sections isolated from the same human lung specimen it was discovered that, whereas the tissue samples exposed to atopic asthmatic serum (SE) displayed markedly induced FcεRII expression relative to control (CO) serum-treated samples, the upregulation of FcεRII expression was largely inhibited by pretreatment of the atopic serum-sensitized tissues with anti-CD23 monoclonal antibody (SE^+). Thus, anti-CD23 MAb significantly attenuated the induction of FcεRII mRNA expression at 24 hours in atopic asthmatic-serum sensitized airway smooth muscle. Moreover, in separate complimentary experiments examining the effects of exogenous administration of IgE immune complexes on FcεRII expression in cultured ASM cells, it was found that treatment of the cells with IgE immune complexes induced progressive enhancement of FcεRII mRNA expression (Figure 8A), resulting in a near 5-fold increase in FcεRII expression at 24 hours (Figure 8B). Additionally, in concert with the above ASM tissue studies, pretreatment of cells with anti-CD23 monoclonal antibody significantly inhibited (i.e., by ~40%) the magnitude of the IgE immune complex-induced FcεRII expression at 24 hours (Figure 8B). Thus, a progressive induction of FcεRII mRNA expression up to 24 hours following treatment with IgE immune complexes, and inhibition of FcεRII expression at 24 hr in the presence of anti-CD23 MAb (hatched bar) was observed.

Taken together, the above observations demonstrate that rabbit and human ASM express mRNA specific for the low-affinity FcγRIII and FcεRII receptors for IgG and IgE, respectively, and that the expression of the FcεRII receptor mRNA, as

examined using RT-PCR, is upregulated by treatment with either human atopic asthmatic serum or exogenously administered IgE immune complexes, secondary to activation of the endogenously expressed FcεRII receptor.

Expression of FcγRIII and FcεRII cell surface proteins in sensitized airway smooth muscle

Given the above findings, the issue of whether ASM cells express FcγRIII and FcεRII receptor proteins on their cell surface was examined by flow cytometry. As shown in Figure 9, ASM cells expressed both the low-affinity FcγRIII (Figure 9A) and FcεRII (Figure 9B) receptors as surface protein. In contrast to unaltered FcγRIII receptor expression after exposure of the cells for 24 hours to control or atopic asthmatic serum (Figure 9A), cell surface expression of the FcεRII receptor was increased by > 2-fold in the atopic asthmatic serum-treated vs. control serum-treated cells (Figure 9B). Thus, rabbit ASM cells expressed surface protein specific for both FcγRIII and FcεRII receptors. In contrast to FcγRIII receptor expression which was unaltered in the presence of atopic asthmatic serum, expression of the FcεRII receptor was increased by > two-fold (i.e., from 17 to 36% in the presence of atopic asthmatic serum.

To further substantiate the above observations, sensitized ASM cells and tissue were subsequently examined for evidence of altered FcεRII receptor expression in by immunofluoresence staining. As shown in Figure 10, relative to an isotype negative control antibody (upper panels: A and B), FcεRII staining with an anti-CD23 antibody was weakly positive both in control (CO) serum-treated cells and tissue (lower left panels: A and B). Moreover, in parallel with the above results based on flow cytometric analysis, surface staining for the CD23 receptor protein was notably enhanced in the atopic asthmatic serum-treated cells and tissue (Figure 10; lower right panels: A and B). Thus, significantly enhanced FcεRII (CD23) receptor staining in ASM cells and tissue treated with atopic asthmatic serum (i.e., lower SE panels in Panel A and Panel B) was observed.

Previous studies have implicated both mast cells and other pro-inflammatory cells in the pathogenesis of asthma. The present study addressed the hypothesis that the resident airway smooth muscle itself also plays an etiologic role in the pathobiology of the disease. The results provide compelling new evidence demonstrating that: 1) ASM cells express mRNA and surface protein for the low-affinity FcγRIII (CD16) and FcεRII (CD23) receptors for IgG and IgE, respectively; 2) in contrast to the FcγRIII receptor, expression of the FcεRII receptor is significantly upregulated following treatment with either atopic asthmatic serum or IgE immune complexes, a finding similar to that obtained in a passively sensitized human ASM; 3) the induced changes in FcεRII receptor expression are associated with altered ASM responsiveness to muscarinic/cholinergic and β-adrenoceptor activation in the atopic asthmatic sensitized state; and 4) the latter changes in FcεRII receptor expression and responsiveness in atopic asthmatic sensitized ASM can be attributed to activation of intrinsically expressed FcεRII receptors by immune complexed IgE.

The use of passive sensitization of rabbit and human isolated airways with human atopic asthmatic serum provides a practical *in vitro* experimental approach to examine the regulation of airway responsiveness in the atopic asthmatic state (Hakonarson et al., 1997, J. Clin. Invest. 99:117-124; Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). In this connection, the observed changes in responsiveness in the atopic sensitized tissues closely mimicked the perturbations in airway function that characterize the *in vivo* asthmatic condition, including exaggerated bronchoconstrictor responsiveness to contractile agonists and impaired airway relaxation to β-adrenoceptor stimulation (Hakonarson et al., 1997, J. Clin. Invest. 99:117-124; Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). When the mechanistic link between exposure of naive airway tissue to atopic asthmatic serum and its resultant altered responsiveness was previously addressed using the same experimental model described herein, it was observed that the latter effect was largely mediated by the induced autologous expression and autocrine action of the cytokine, IL-1β, in atopic sensitized ASM

(Hakonarson et al., 1997, J. Clin. Invest. 99:117-124). Moreover, the mechanism of action of IL-1β in eliciting the altered responsiveness in sensitized tissues was attributed to its induced upregulated expression of G_i protein, specifically $Gi\alpha_2$ and $Gi\alpha_3$ which inhibit intracellular cAMP accumulation (Hakonarson et al., 1996, J. Clin. Invest. 97:2593-2600).

The data presented herein establish that the sequence of events leading to cytokine-induced changes in ASM responsiveness in the sensitized state is initiated by IgE-coupled FcεRII receptor activation involving the ASM itself. The present observations demonstrate that either depletion of serum immune complexes or pretreatment with a specific anti-CD23 monoclonal blocking antibody largely prevented the changes in ASM responsiveness induced by exposure of the tissues to atopic asthmatic serum (Figures 1 and 2). Furthermore, in complimentary studies, exposure of naive ASM tissue to IgE immune complexes produced changes in ASM responsiveness (Figures 3 and 4) which closely resembled those observed in the atopic sensitized state. Thus, IgE-coupled Fc receptor activation principally involving the FcεRII receptor in ASM itself appears to be fundamentally responsible for producing the perturbations in airway responsiveness that characterize the pro-asthmatic state.

In concert with the above pharmacodynamic results, additional experiments demonstrated the presence of constitutive FcεRII and FcγRIII receptor mRNA and cell surface expression, both in cultured ASM cells and human ASM tissue (Figures 5 and 6). Furthermore, in contrast to FcγRIII expression, which was unaltered in the presence of atopic asthmatic serum, expression of FcεRII was markedly enhanced in ASM exposed to the sensitizing atopic serum (Figures 5 and 6). In considering these results, it is relevant to note that a number of studies have reported a direct correlation between serum IgE levels and FcεRII and -RI expression (Spiegelberg et al., 1981, J. Clin. Invest. 68:845-852; Conroy et al., 1977, J. Immunol. 118:1317-1321; Malveaux et al., 1978, J. Clin. Invest. 62:176-181). While these reports raised the notion of a possible common mechanism regulating the production of both IgE and its receptors, more recent evidence based on studies using different cell

lines suggests that IgE itself may enhance FcεRI (Lantz et al., 1997, J. Immunol. 158:2517-2521; Yamaguchi et al., 1997, J. Exp. Med. 185(4):663-672) or FcεRII expression (Lee et al., 1987, J. Immunol. 139:1191-1198; Daeron et al., 1986, J. Immunol. 136:1612-1619; Lee et al., 1986, J. Immunol. 136:4573-4580). The latter concept is supported by the present experiments, wherein it was observed that the induced expression of FcεRII in ASM treated with atopic asthmatic serum was largely inhibited in the presence of an anti-CD23 blocking antibody (Figure 7). Moreover, as further support for the concept of IgE-dependent modulation of FcεRII expression, it was also observed that exogenously administered IgE immune complexes elicited an increase in FcεRII expression (Figure 8A) and, further, that this effect was significantly inhibited by pretreatment with anti-CD23 MAb (Figure 8B). Thus, the data presented herein are consistent with the presence of a positive feedback system in ASM wherein IgE upregulates the expression of its own low affinity receptor. This notion is in general agreement with previous studies which reported the presence of IgE-dependent Fcε receptor induction in other cell types (Lantz et al., 1997, J. Immunol. 158:2517-2521; Yamaguchi et al., 1997, J. Exp. Med. ??:663-672; Lee et al., 1987, J. Immunol. 139:1191-1198; Daeron et al., 1986, J. Immunol. 136:1612-1619; Lee et al., 1986, J. Immunol. 136:4573-4580). However, in these earlier studies mRNA levels for Fcε receptor expression were not examined, and the increased cell surface expression of the receptor was attributed to IgE-mediated inhibition of proteolytic cleavage of the receptor from its membrane binding site (Lee et al., 1987, J. Immunol. 139:1191-1198). Recognizing the limitations in quantitative analysis using RT-PCR, the present observations demonstrating the presence of IgE-dependent enhanced expression of FcεRII mRNA suggest that IgE exerts its upregulatory action on FcεRII expression, at least in part, via a pre-translational effect.

In recent years, the complexity of transmembrane signaling via the FcεRII receptor has been partially unraveled. In this regard, together with IgE binding, signaling via the FcεRII receptor is known to include coupling of the receptor to complement receptor 2 (CR2), also referred to as CD21, which is a receptor for

fragments of the complement component, C3 (Aubry et al., 1992, Nature 358:505-507). Further, as demonstrated in B-lymphocytes, CR2 co-ligates with the membrane protein, CD19 and, in the presence of C3 fragments bound to antigen/immune complexes (e.g., IgE/IgG and/or IgM), this membrane-linked network of molecules acts synergistically to mediate the cytokine activities of FcεRII (Sutton et al., 1993, Nature 366:421-428; Delespesse et al., 1992, Immunol. Rev. 125:77-97; Matsumoto et al., 1991, J. Exp. Med. 173:55-64). Accordingly, in B-lymphocytes, this antigen/IgE immune complex network coupled to the FcεRII receptor apparently serves to facilitate antigen presentation to antigen-specific CD4+ (helper) T-lymphocytes, a process resulting in the expression of the CD4+/Th2 phenotype. The latter phenomenon is associated with the release of such Th2-derived cytokines as IL-4 and IL-5 which are involved in B-lymphocyte switching to IgE production and eosinophil accumulation, respectively (Delespesse et al., 1992, Immunol. Rev. 125:77-97). With potential implications related to asthma, recent evidence based on studies conducted in house dust mite-immunized mice suggests that the above mechanism of IgE/FcεRII-coupled facilitation of antigen presentation to T-lymphocytes is important for both the induction of the Th2-type immune response in the lungs and the subsequent infiltration of eosinophils into the airways following inhaled antigen challenge (Coyle et al., 1996, J. Exp. Med. 183:1303-1310).

In light of the above evidence regarding the role of CD23 receptor activation in eliciting Th-2 cytokine production and pulmonary eosinophilic infiltration in the antigen-sensitized state, the results of the present study raise the consideration that, apart from inducing changes in airway responsiveness, upregulated FcεRII expression and activation in airway smooth muscle cells may also play a role in modulating local airway immune responses. Indeed, this compelling concept is in part supported by the recent observation that airway smooth muscle cells are autologously induced to release IL-1β in the atopic asthmatic sensitized state (Hakonarson et al., 1997, J. Clin. Invest. 99:117-124), a finding which, together with the present observations, suggests the presence of FcεRII-coupled local stimulation of cytokine

release and signaling. In this context, it is important to determine whether IgE-coupled activation of FcεRII receptors in airway smooth muscle can also induce the release of Th-2-like cytokines and, thereby, promote local infiltration of the airways with eosinophils and other proinflammatory cells seen in the asthmatic state.

In conclusion, the present study has identified the presence and up-regulated expression of FcεRII receptors in atopic asthmatic-sensitized airway smooth muscle, and has further demonstrated that the latter phenomenon is associated with the induction of altered airway responsiveness in the atopic asthmatic sensitized state. While comparably up-regulated expression of the FcεRII receptor has been demonstrated in different bone marrow-derived circulating leukocytes in atopic asthmatic individuals (Williams et al., 1992, J. Immunol. 149:2823-2829; Gagro et al., 1993, Int. Arch. Allergy Immunol. 101:203-208; Rabatic et al., 1993, Exp. Immunol. 94:337-340; Joseph et al., 1983, J. Clin. Invest. 71:221-230), the present observations raise a novel consideration regarding the pathobiology of asthma.

Accordingly, given the important immunological and proinflammatory responses attributed to FcεRII receptor activation, the present findings identify an important role and mechanism for the resident airway smooth muscle in autologously inducing its own IgE-mediated state of altered responsiveness contributing to the atopic asthmatic condition.

The disclosures of each and every patent, patent application and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed is:

1. A method of preventing induction of an asthmatic state in a human patient comprising administering to said human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby preventing induction of said asthmatic state in said human.

2. The method of claim 1, wherein said ligand is selected from the group consisting of an isolated protein , an isolated polypeptide and a non-peptide.

3. The method of claim 2, wherein said ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody.

4. The method of claim 3, wherein said anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.

5. The method of claim 1, wherein said ligand is administered to said human in the form of an isolated DNA encoding and capable of expressing said ligand.

6. The method of claim 5, wherein said DNA is formulated in a viral or a non-viral vector.

7. The method of claim 6, wherein said viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus.

8. The method of claim 7, wherein said non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.

9. The method of claim 1, wherein said anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.

10. The method of claim 1, wherein said pharmaceutically acceptable carrier is physiological saline.

11. A method of treating asthma in a human patient comprising administering to said human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby treating asthma in said human.

12. The method of claim 11, wherein said ligand is selected from the group consisting of an isolated protein, an isolated polypeptide and a non-peptide.

13. The method of claim 12, wherein said ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody.

14. The method of claim 13, wherein said anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.

15. The method of claim 11, wherein said ligand is administered to said human in the form of an isolated DNA encoding and capable of expressing said ligand.

16. The method of claim 15, wherein said DNA is formulated in a viral or a non-viral vector.

17. The method of claim 16, wherein said viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus.

18. The method of claim 17, wherein said non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.

19. The method of claim 11, wherein said anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.

20. The method of claim 21, wherein said pharmaceutically acceptable carrier is physiological saline.

21. A method of treating a human having asthma comprising administering to said human a pharmaceutically effective amount of an isolated nucleic acid encoding an anti-FcεRII receptor protein ligand, wherein said nucleic acid expresses said ligand in vivo in an amount sufficient to treat said asthma.

22. A method of identifying an anti-FcεRII receptor protein ligand comprising

providing a mixture comprising IgE and a population of cells which express an FcεRII receptor protein,

incubating said mixture in the presence or absence of a test ligand, and

measuring the level of IgE bound to said FcεRII receptor protein, wherein a lower level of IgE bound to said cells in the presence of said test compound compared with the level of binding of IgE to said cells in the absence of said test compound is an indication that said test compound is an anti-FcεRII receptor protein ligand.

23. The method of claim 22, wherein said cells are airway smooth muscle cells.

24. A ligand useful for inhibiting binding of IgE to an FcεRII receptor protein identified using the method of claim 22.

25. A method of inhibiting binding of IgE to an FcεRII receptor protein expressed on a cell comprising administering to said cell an anti-FcεRII receptor protein ligand.

ABSTRACT OF THE INVENTION

Methods of treating or preventing induction of asthma in a human patient are provided. The methods comprise administering to the human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby treating or preventing induction of asthma in the human.

HALONAGEN & GRANSTAM

Figure 1.

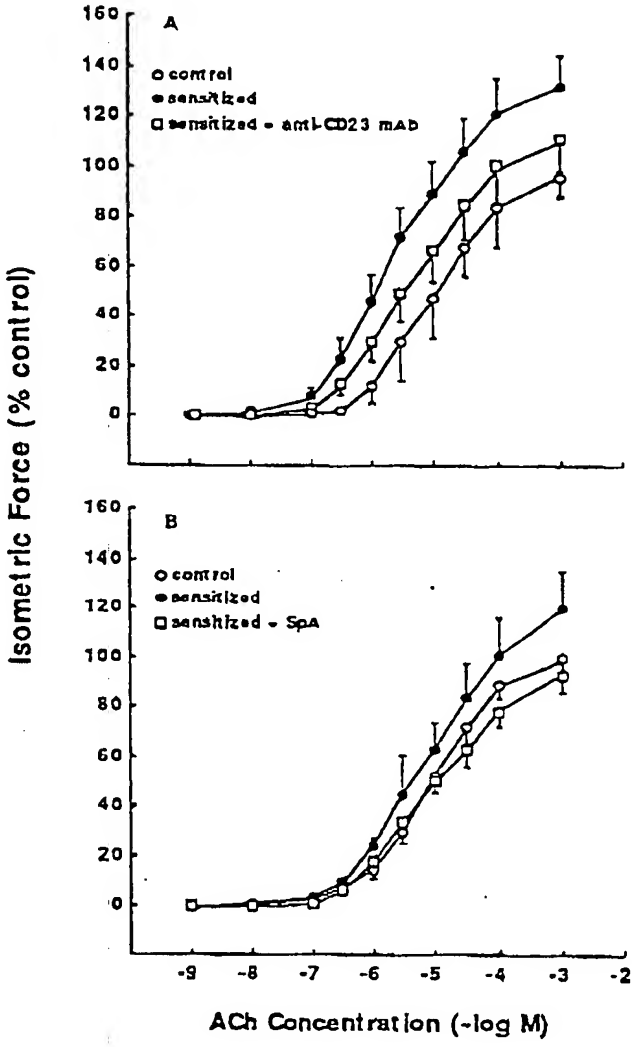
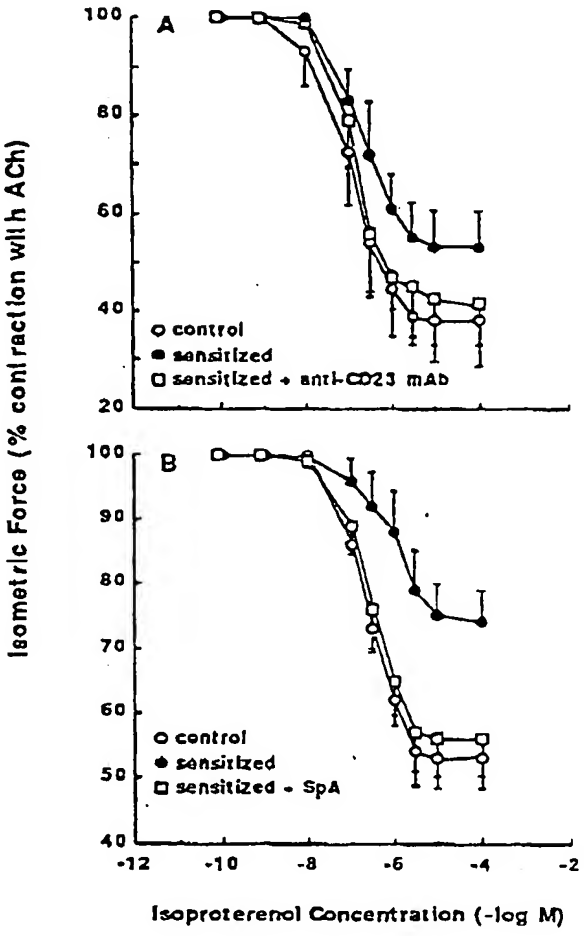


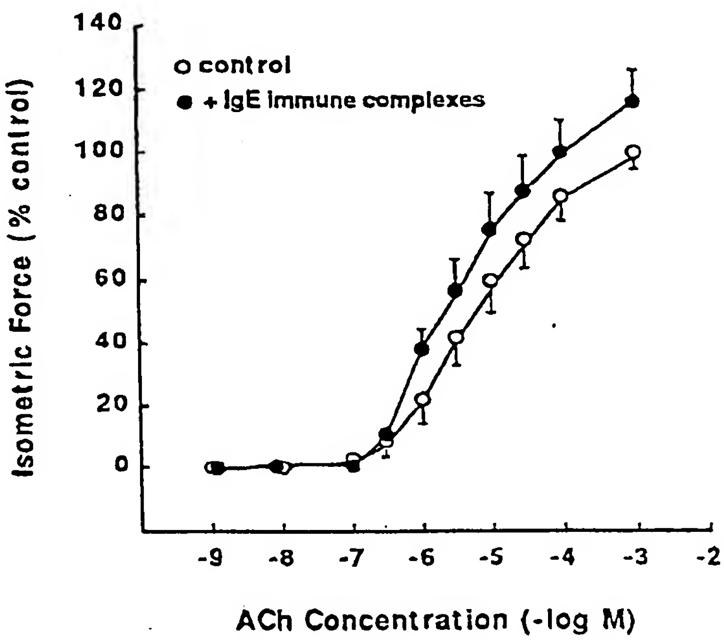
Figure 2.

HARLOW/HARLOW & BRUNSTEIN



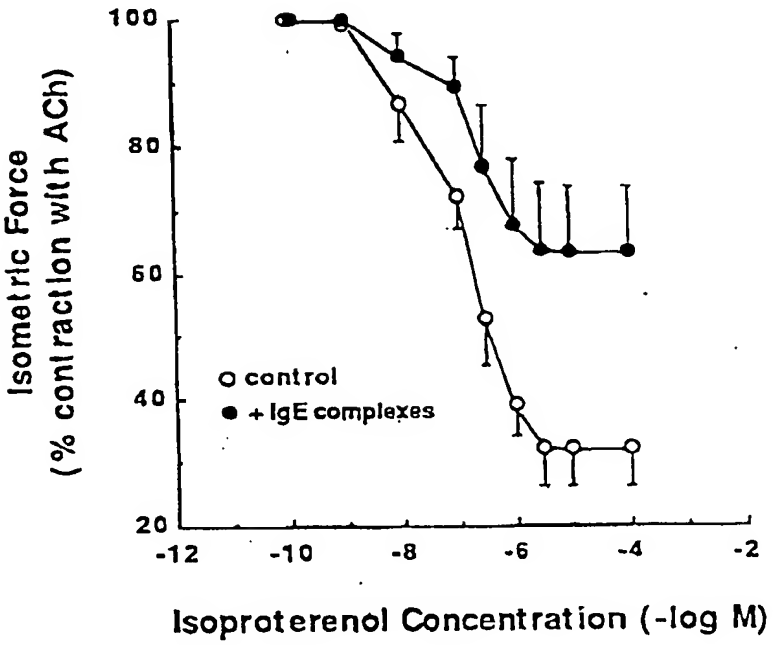
Hallgrimsson & Grunstein

Figure 3



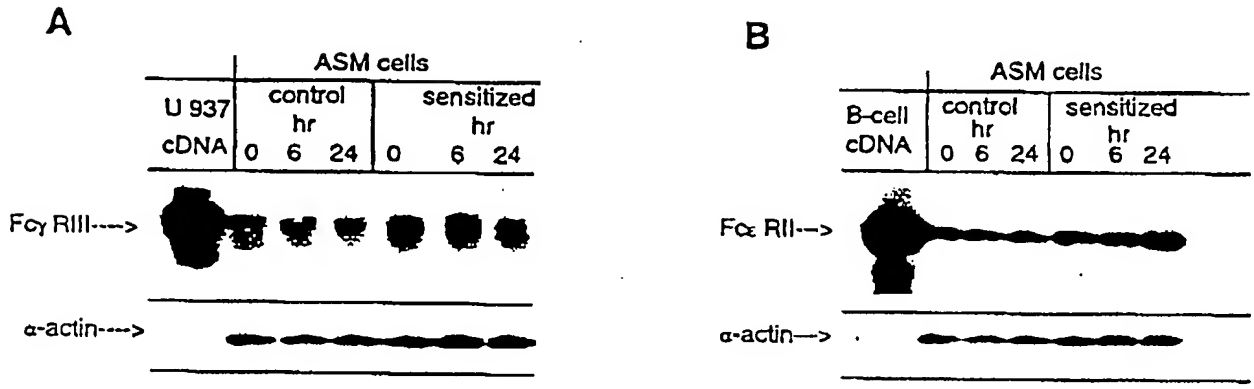
Hammarsten & Bernstein

Figure 4.



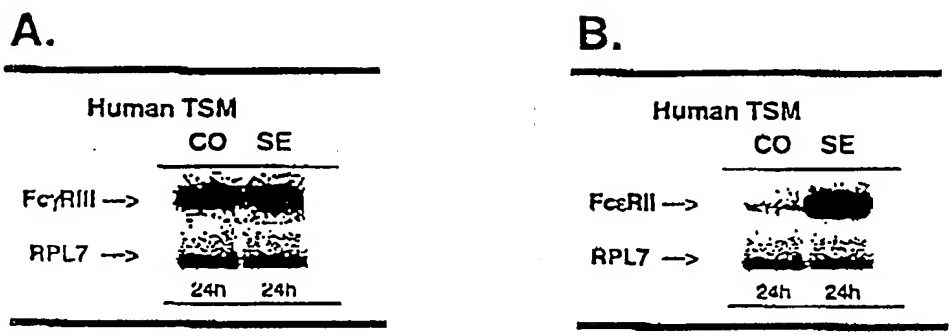
Hamorrson & Bernstein

Figure 5.



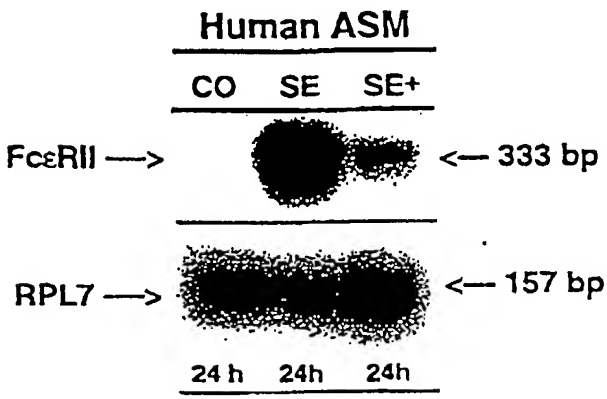
HALONEN SON & GENETON

Figure 6



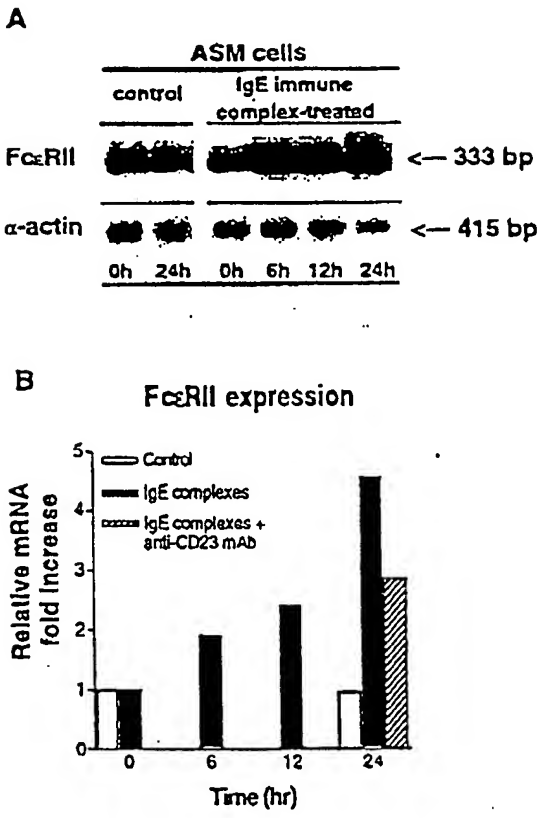
Hammond & Bernstein

Figure 7.



HAMMERSON & GOLDSTEIN

Figure 8.

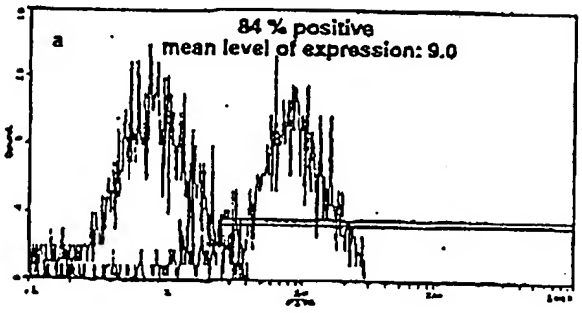


HALONARSON & GRUNSTEIN

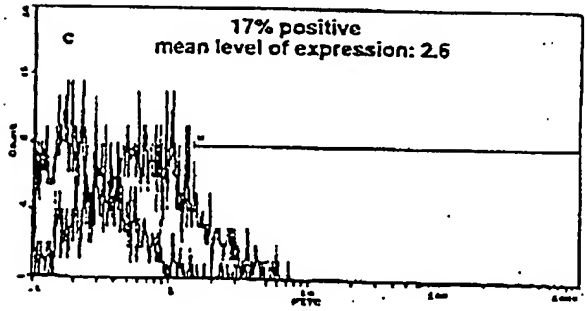
Figure 9.

A. Control serum

FcγRIII

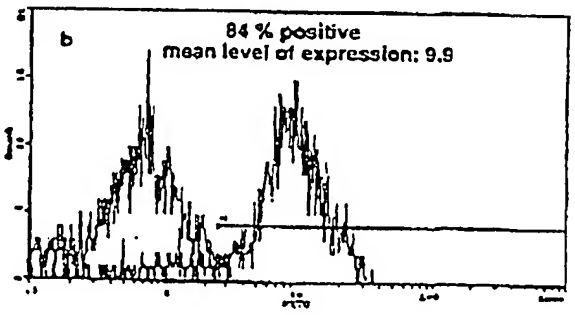


FcεRII



B. Atopic/asthmatic serum

FcγRIII



FcεRII

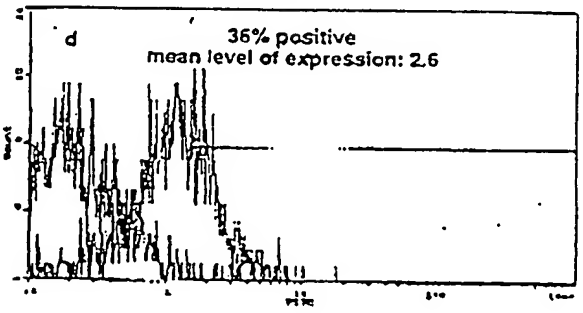
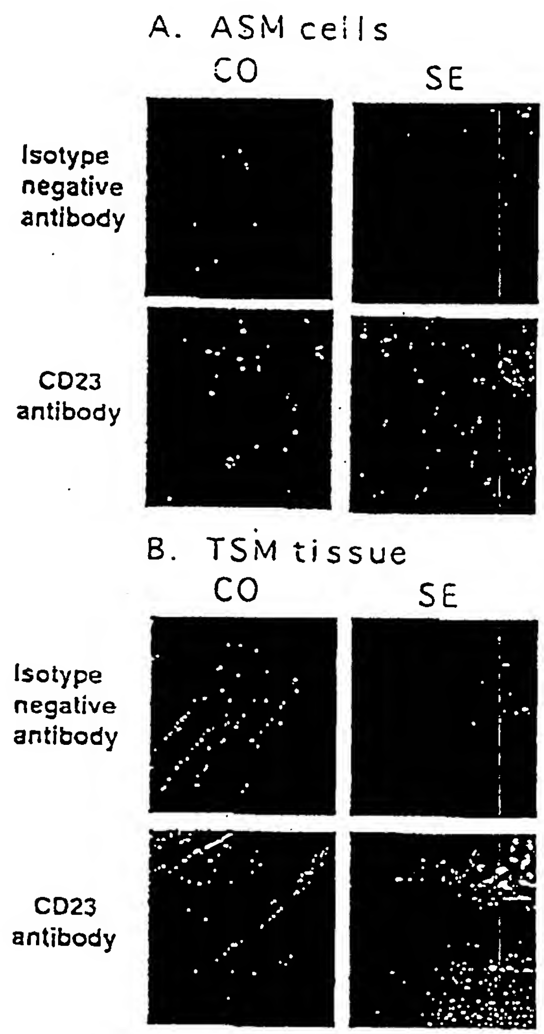


Figure 10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CO
SE
Isotype negative antibody
CD23 antibody
B. TSM tissue
CO
SE
Isotype negative antibody
CD23 antibody

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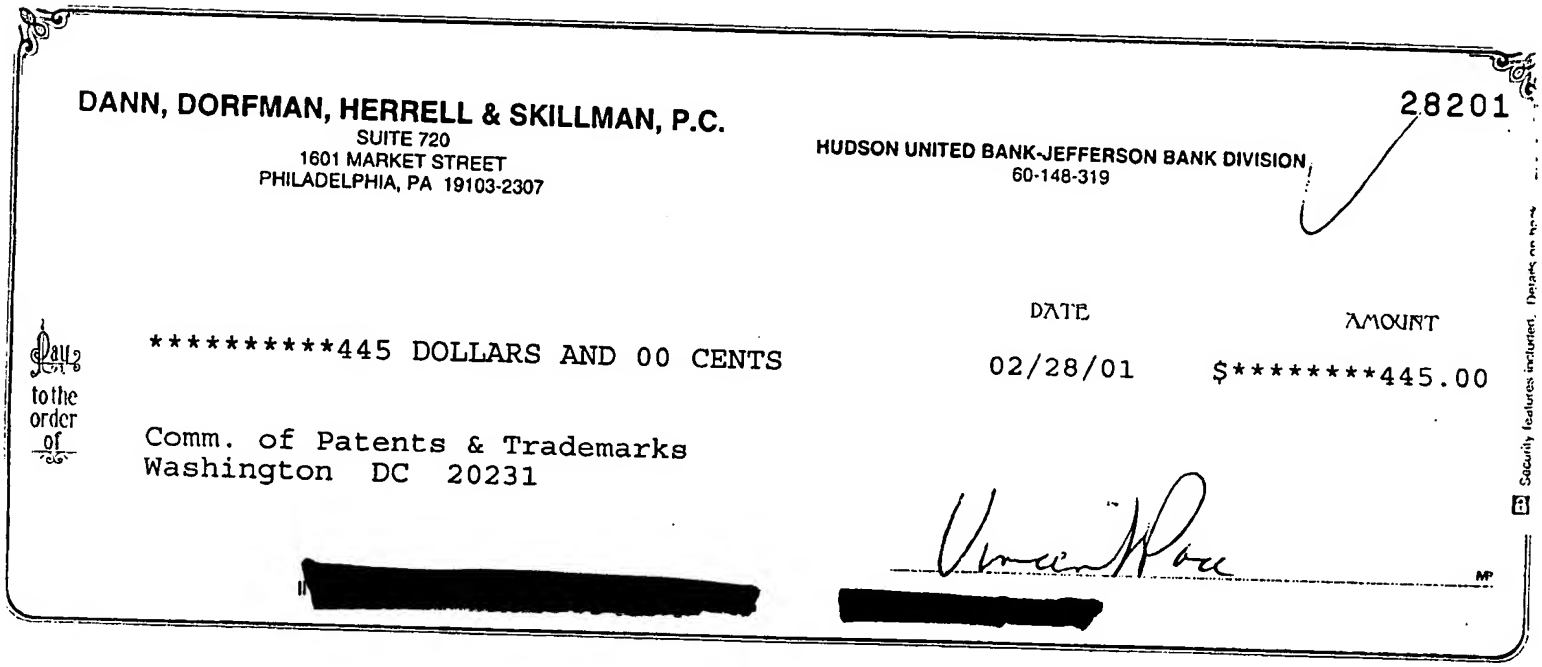

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